CEREAL CHEMISTRY

Published bi-monthly by

The American Association of Cereal Chemists

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Entered as second class matter at the post office at Minneapolis, Minn., under the Act of August 24, 1912. Acceptance for mailing at special rate of postage provided for in paragraph (d-2), Section 34.40, P. L. & R. of 1948, authorized February 16, 1924. Published at 500 So. 5th St., Minneapolis, Minn.

VOL. XXXI MARCH, 1954 No. 2

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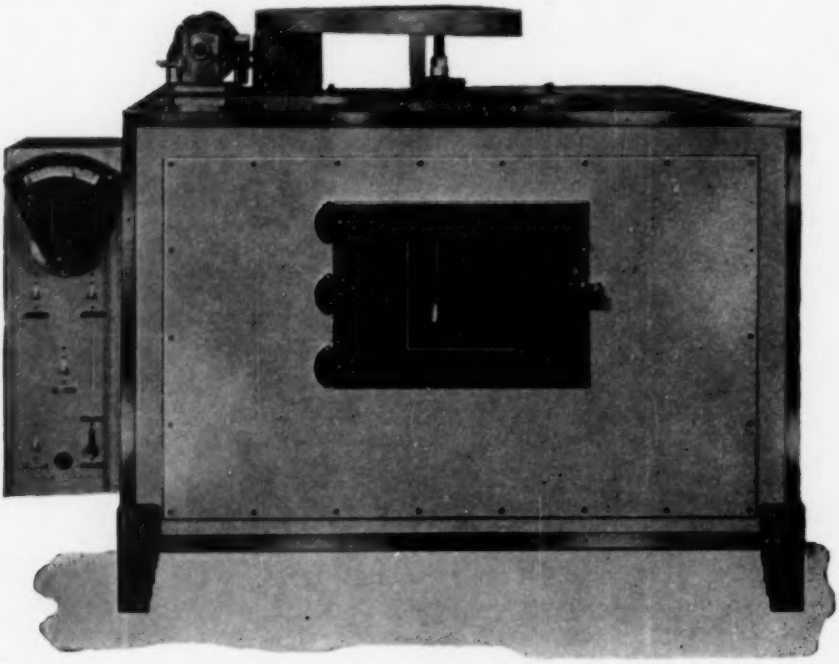

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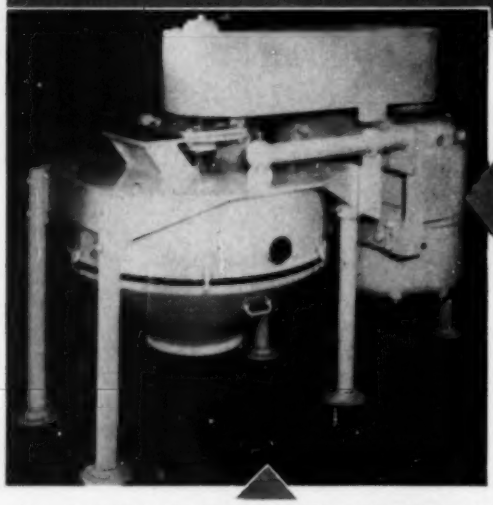

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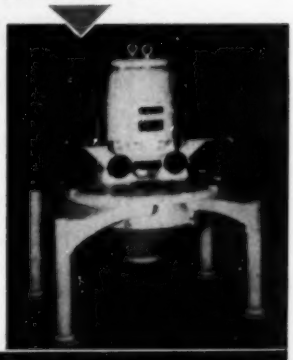

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White Flour in the U.S.A.—

Enriched* with Vitamins and Iron for Better Nutrition

by Science Writer

This article is one of a series devoted to the enrichment of family white flour, white bread and rolls, corn meal and grits, macaroni products and white rice.

The enrichment of family white flour with thiamine, riboflavin, niacin and iron by American millers is a major success. It has made great contributions to better health in the United States by improving a staple food.

Since the start of the program in 1941 by millers, voluntarily, the evidence is conclusive that enrichment not only has reduced the number of cases of certain dietary diseases but also is promoting the mental and physical vigor and well-being of the U. S. population generally. Because of its demonstrated value, the principle and practice of enrichment have been applied to other foods made from grains: corn meal and grits, white rice, macaroni, spaghetti, noodles, pastina, farina—and, of course, to white bread and rolls.

Doctors and diet experts have long supported white flour enrichment. The Council on Foods and Nutrition of the American Medical Association and the Food and Nutrition Board of the National Research Council are on record as endorsing the practice.

The legislatures of a majority of the States plus Hawaii and Puerto Rico have enacted laws which make mandatory the enrichment of all family white flour, as well as white bread, sold commercially in those areas.

American homemakers, too, favor foods they know to be enriched—a fact demonstrated by surveys. They look for these words on package labels: "Enriched with vitamins and iron for better nutrition."

What Is Enrichment?

It's an axiom in the milling industry that consumers want beautifully fine, white flour. When wheat is milled and processed to get the white flour which the public demands, vitamin and mineral values are unavoidably lost.

Enrichment restores to white flour the following important vitamin and mineral factors: thiamine, riboflavin, niacin and iron. Calcium also may be added as an optional ingredient. The process is simple and inexpensive. A mixture of the vitamins and iron is fed into the flour stream during processing. This insures that the enriching ingredients are spread evenly throughout the flour.

The U. S. Food and Drug Administration has established standards which white flour must meet to be properly labeled enriched. The requirements, in milligrams per pound, are:

	Min.	*Max.*
Thiamine (vitamin B_1)	2.0	2.5
Riboflavin (vitamin B_2)	1.2	1.5
Niacin (another "B" vitamin)	16.0	20.0
Iron	13.0	16.5

(In Canada, too, the same standards have been set for enriched white flour through the amendment of the Food and Drugs Act.)

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The science of chemistry is so advanced these days that many of Nature's complex substances can be "duplicated" in the laboratory. This has happened with many vitamins. First, the chemical composition is learned. Second, the pure substance is isolated. Third, a "duplicate" is made by synthesis. And fourth, the laboratory techniques are extended to large scale operation.

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Reprints of this article, and all others in this series are available without charge. Please send your request to the Vitamin Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada: Hoffmann-La Roche Ltd., 286 St. Paul Street, West; Montreal, Quebec.

The watermills are gone. Today's needs require today's methods. How sensible it is that millers across the nation restore health-giving vitamins and minerals through enrichment.

*Webster's Merriam Collegiate Dictionary includes this definition of "enrich": "to improve (a food) in nutritive value by addition in processing of vitamins and minerals".

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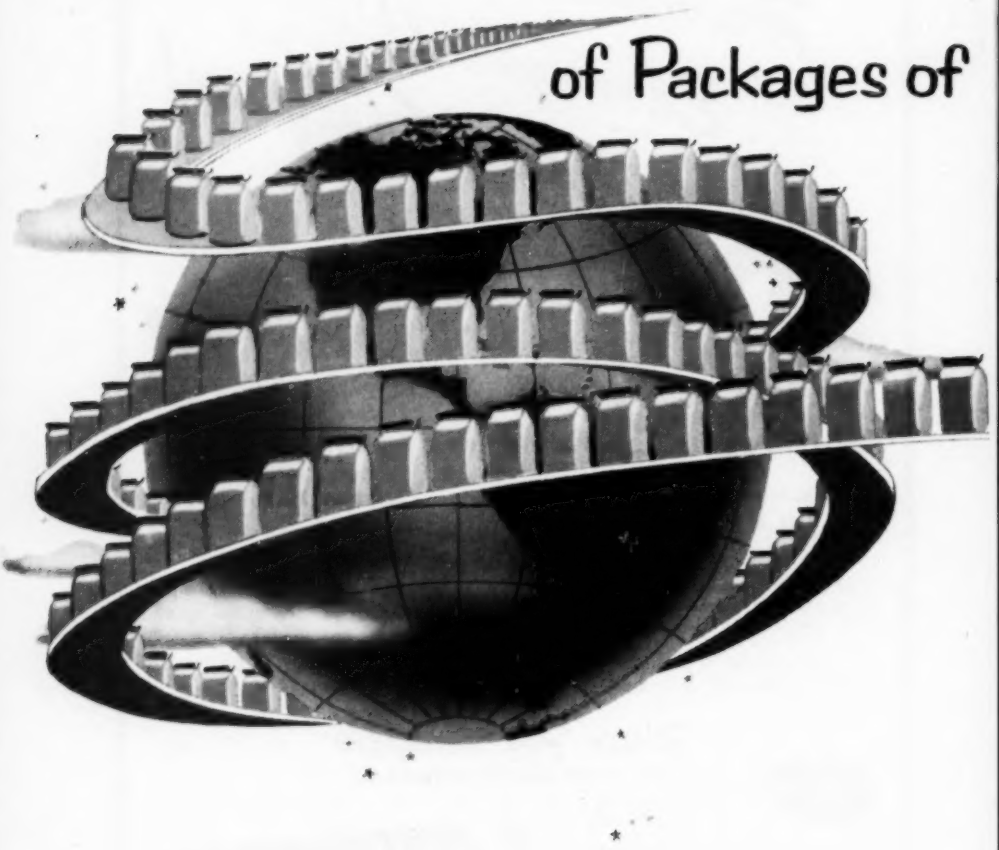

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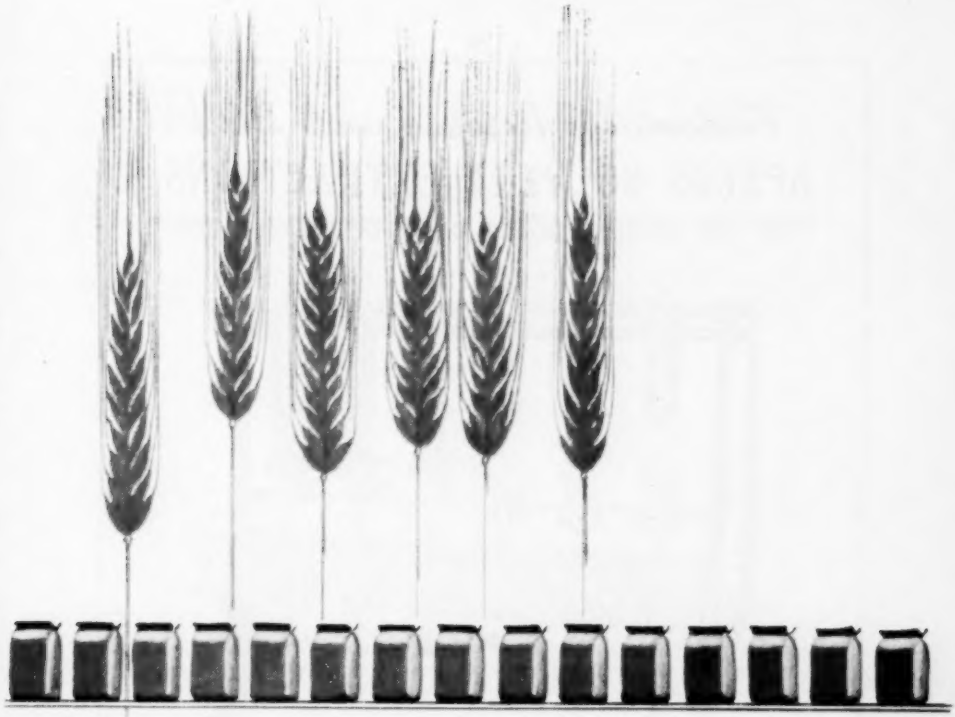

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CEREAL CHEMISTRY

VOL. XXXI MARCH, 1954 NO. 2

THE FATE OF SUGARS IN BREAD DOUGHS AND SYNTHETIC NUTRIENT SOLUTIONS UNDERGOING FERMENTATION WITH BAKER'S YEAST[1]

R. B. KOCH,[2] F. SMITH, AND W. F. GEDDES

ABSTRACT

Paper partition chromatography has been applied to the separation and determination of the amounts of individual sugars present in bread doughs, bread, and synthetic nutrient solutions undergoing fermentation with a commercial baker's yeast which had been cultivated in a molasses medium. Sucrose was hydrolyzed very rapidly; even in straight doughs made with 3% yeast and 7.5% sucrose, inversion was complete when the first samples were analyzed (7 minutes after mixing). Glucose was fermented at a uniform and rapid rate, either alone or in mixtures with other sugars. Glucose exerted a sparing action on the fermentation of fructose; when glucose was absent or present only in low concentrations, fructose was fermented at a similar rate to that of glucose. As a consequence, bread made with sucrose contained a higher percentage of fructose than glucose.

When glucose, fructose, and maltose were present in a fermenting medium, maltose was fermented at a slow rate until the glucose and fructose were exhausted. At this point there was a temporary decrease in the rate of carbon dioxide production, but the yeast soon developed the ability to ferment maltose rapidly and a second maximum in gas production was attained. When baker's yeast became adapted to fermenting maltose, the addition of glucose did not prevent a rapid decrease in maltose fermentation.

For sponge and dough fermentations, there is greater utilization of maltose by the yeast than in straight doughs, and this is reflected in lower maltose content of bread baked by the sponge process.

Although yeast fermentation has been extensively studied by many investigators, there is little precise knowledge concerning the rate at which various sugars are fermented in breadmaking, or of the nature of the residual sugars in bread made by different formulas and baking procedures. The comprehensive review of yeast fermentation by Atkin, Schultz, and Frey published in 1946 (3) reveals that there are a number of variables which influence panary fermentation. The sugars in bread doughs arise from three sources: the sugars originally present in the flour, sugars produced from oligosaccharides or polysaccharides

[1] Manuscript received August 26, 1953; presented at the annual meeting, May, 1952. Contribution from the Department of Agricultural Biochemistry, University of Minnesota, Saint Paul, Minnesota. Paper No. 3049 of the Scientific Journal Series, Minnesota Agricultural Experiment Station. The data in this paper are taken from a thesis presented to the Graduate School of the University of Minnesota by R. B. Koch in partial fulfillment of the requirements for the Ph.D. degree, March, 1952.

[2] Present address: J. R. Short Milling Co., Chicago 9, Ill.

by the action of flour and yeast enzymes, and those intentionally added as dough ingredients.

The strains of yeast, *Saccharomyces cerevisiae,* which have been selected and propagated for use in bread manufacture possess high fermenting power. Glucose is fermented at a slightly faster rate than fructose (7, 8, 9, 13, 16, 25). As sucrose is hydrolyzed by yeast much more rapidly than it is fermented (6), its rate of fermentation is equivalent to that of an equimolecular mixture of glucose and fructose. Baker's yeast cultivated on molasses has a much longer induction period for maltose than for other sugars (3, 4, 8), although the induction period is considerably shortened in the presence of glucose, sucrose, maltase, and oxygen (15, 20, 21). Lanning (12), Larmour and Bergsteinsson (13), and Larmour and Brockington (14) have shown that maltose is fermented in a dough after glucose, fructose, and sucrose.

Few investigations of the sugar content of bread have been reported in the literature. The most significant study was conducted by Rice, who determined the individual sugars in bread made from known formulas employing special fermentation and copper reduction procedures (17). He found a higher content of fructose than of glucose in breads made from a formula containing sucrose. The maltose content of breads made by the sponge and dough process was lower than for those made by straight dough procedures with corresponding additions of sugars.

The interpretation of changes in the concentration of different sugars in bread doughs is complicated by the production of fructose from polyfructosans and of maltose from starch. The use of synthetic nutrient solutions has made it possible to study the fermentation of individual sugars, both singly and combined under controlled conditions. Atkin, Schultz, and Frey (3) and, more recently, Rice and Ramstad (18) have reviewed the extensive literature in this field.

In separate solutions and within certain ranges of concentration, glucose and fructose are fermented at the same rate (9, 22–25). Slator (22), Hopkins (7, 8), and Hopkins and Roberts (9) have shown that brewer's yeasts ferment glucose more rapidly than fructose in mixtures of the two sugars. Reference has already been made to the rapid inversion of sucrose by baker's yeast and to the long induction period for maltose fermentation when the yeast is grown on molasses. It has been shown that the enzymes responsible for the fermentation of maltose are adaptive and are formed only after the yeast cell has been in contact with the sugar (19). In contrast, the enzymes responsible for the fermentation of sucrose are constitutive and hence the relative

rates of the fermentation of sucrose and maltose depend upon the yeast and a number of other factors.

Until recently the analyses of complex mixtures of the various sugars to be found in bread doughs could be accomplished only by the use of special yeasts in conjunction with determinations of reducing sugars. During the last few years, however, chromatographic technics have been perfected which permit the separation and quantitative determination of the various sugars. In a previous communication the authors (10) have shown how chromatographic analyses may be used to provide precise information concerning the sugars of wheat flour. The present paper deals with the determination of individual sugars in fermenting doughs and bread made with various sugars. The majority of the experiments were conducted on straight doughs, but sugar determinations were carried out on a sponge and dough prepared in the laboratory by a commercial-type formula and also on samples obtained from a commercial bakery employing a sponge and dough procedure. Experiments were also conducted on the rates of fermentation and gas production in nutrient solutions containing different sugars.

Materials and Methods

Preparation of Experimental Doughs and Bread. In making the experimental doughs, a southwestern baker's patent flour (protein 11.8%, ash 0.43% at 14.0% moisture) was used unless otherwise stated. In one series of experiments, doughs were made with a baker's patent flour (protein 14.2%, ash 0.44%) milled from hard red spring wheat.

Doughs and breads were prepared in three ways, namely: a straight dough procedure employing the A.A.C.C. basic formula (1), a straight dough commercial-type formula, and a sponge and dough commercial-type formula.

Ingredient	*Straight Dough Formula*		*Sponge and Dough Formula*	
	Basic	*Commercial*	*Sponge*	*Dough*
	g.	g.	g.	g.
Flour (14.0% moisture basis)	100.0	100.0	70.0	30.0
Yeast, compressed[a]	3.0	3.0	2.0	...
Salt	2.0	2.0	...	2.0
Sugars	variable	variable	...	variable
Malt	...	...	0.5	...
Nonfat milk solids	...	3.0	...	5.0
Shortening, hydrogenated	...	3.0	...	3.0
Potassium bromate	0.001	0.001	...	...
Yeast food	...	...	0.5	...
Water	as required	as required	as required	as required

[a] The commercial yeast used in these experiments was cultivated in a molasses medium.

For each straight-dough formula, twice the quantities shown were mixed for 3 minutes in a Hobart mixer equipped with a McDuffee bowl, the temperature of the water being adjusted to yield doughs of 30°C. After mixing, the dough was divided into two equal parts and fermented and proofed at 30°C. and 85 to 90% relative humidity. The doughs were given a first punch 95 minutes after mixing and were molded and panned after an additional 25 minutes' fermentation. After proofing for 60 minutes, the doughs were baked at 232°C. for 25 minutes. In the sponge and dough procedure double the quantities of the various ingredients were also employed and the dough was divided after mixing. The sponge ingredients were mixed in the McDuffee bowl of a Hobart-Swanson mixer for 1.5 minutes and fermented 4 hours at 30°C. The remaining ingredients were added, and mixed for 5 minutes; the doughs were then scaled into two equal parts, and after 30 minutes' fermentation they were panned, proofed, and baked in the manner previously described.

Commercial Sponges and Doughs. Sponges and doughs were obtained from a large commercial bakery and the sugar levels determined at various stages in the baking process. The formula supplied by the bakery is given below.

Ingredient	*Quantity as % of Flour*	
	Sponge	*Dough*
Flour	65.0	35.0
Yeast, compressed	2.0–3.0	....
Yeast food	0.25–0.50	....
Malt	0.17–0.33	....
Lard	2.0–3.0	....
Sucrose	....	3.0
Glucose	....	3.0
Salt	....	2.0–2.5
Nonfat milk solids	....	3.0
Shortening	....	2.0–3.0
Water	as required	as required

The sponge ingredients were mixed in a high-speed mixer for 5 minutes and fermented in covered troughs for 4 hours in a room maintained at 80°F. (27°C.). The dough was mixed in a high-speed mixer for 15 minutes, fermented 25 minutes at 80°F., passed through the divider, fermented for an additional 10 minutes at 80°F., and then rounded, molded, and panned. The doughs were proofed to height (about 70 minutes) at 105°F. (40.5°C.) and 84% humidity, and then baked for 25 minutes at 450°F. (232°C.) in the instance of the 1.5-lb. loaf.

Sugars Used in the Dough Formulas. The majority of the experiments were conducted with straight doughs prepared by the basic

formula. Doughs were made without added sugars, with three levels of sucrose (2.5, 5.0, and 7.5%), 5.0% glucose, 5.0% fructose, with 4.0% sucrose + 4.0% glucose, and with three types of corn sirup[4], each added at levels to be specified later.

Chromatographic Separation and Quantitative Determination of the Sugars. The residual sugars in the doughs were determined at selected intervals of time during fermentation and also in the bread. This involved extraction of the dough (40 g.) with hot 70% ethanol, heat treatment at 80°C. for 7 minutes to inactivate the enzymes, and dialysis of the extract followed by chromatographic separation and quantitative determination of the sugars in the dialysate by the phenol-sulfuric acid method (5), according to the procedures described by Koch, Geddes, and Smith (13). The amount of water used for dialysis and for extracting the separated sugars from the pieces of filter paper cut from the irrigated chromatogram was adjusted so that a 2.0-ml. aliquot of the extract would contain sufficient sugar to give an absorbance of approximately 0.3.

Yeast Fermentations in a Synthetic Nutrient Solution. A synthetic nutrient solution developed by Atkin, Schultz, and Frey (2) was used to study the relative rates of fermentation of different sugars encountered in breadmaking. In these experiments gas production was also measured.

The following stock solutions were prepared:

Solution A (g./200 ml.)
- Monosodium phosphate monohydrate, 9.6
- Magnesium sulfate heptahydrate, 6.4
- Potassium chloride, 2.56

Solution B (citrate buffer, pH 5.1) (g./1000 ml.)
- Sodium citrate, 100.0
- Citric acid, 20.0

Solution C (mg./25 ml.)
- Thiamine, 27.0
- Pyridoxine, 27.0
- Nicotinic acid, 27.0

Yeast suspension, 6.0 g./100 ml.

For each trial, 0.5 to 1.5 g. of one or more of the sugars under study and 0.2 g. asparagine were placed in a 125-ml. Erlenmeyer flask; 5.0 ml. solution A and 2.4 ml. solution B were added and the mixture warmed slightly to dissolve the sugars and asparagine. After

[4] Paper partition chromatography, coupled with the determination of the sugars by the phenol-sulfuric acid method, showed the sugar content of these sirups to be as follows:

Sugar	Corn sirup number		
	1	2	3
Glucose, %	20.4	20.7	34.6
Maltose, %	19.4	35.7	17.4
Maltotriose, %	13.6	12.9	14.5

cooling to 30°C., 74 μl. solution C and 10 ml. yeast suspension at 30°C. were added and the flask was placed in a water thermostat at 30°C. and clamped to a device for producing 65 to 70 shakes per minute, a rate which was sufficient to keep the yeast in suspension. Each flask was connected by means of rubber tubing to an inverted 100-ml. burette filled with, and immersed in, saturated sodium chloride solution for measuring the carbon dioxide production. The zero time reading was taken about 30 seconds after the yeast was added and the gas volume was read at atmospheric pressure every 15 minutes throughout the trial.

An identical reaction mixture in a second flask was used to follow the change in sugar concentration. An appropriate aliquot was added to 10 ml. of 70% aqueous ethanol contained in a 25-ml. flask immersed in a water bath at 80 to 85°C. After heating with occasional swirling for 10 minutes, the contents were made up to volume at room temperature with distilled water. The individual sugars were separated by partition chromatography and quantitatively determined by the methods outlined in a previous section.

Results and Discussion

Changes in the Concentration of Individual Sugars During Dough Fermentation. Straight-Dough Procedures: The sugar levels at various fermentation times found in straight doughs made by the A.A.C.C. basic formula containing no added sugar, sucrose, glucose, fructose, and sucrose plus glucose, respectively, are shown in Figs. 1 to 7 inclusive.

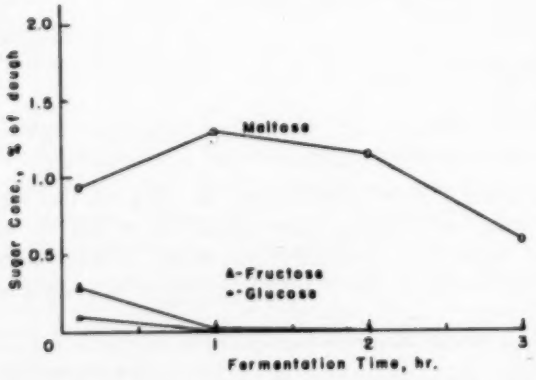

Fig. 1. Changes in sugar concentration during fermentation at 30°C. of a basic straight dough made with 3.0% yeast but no added sugar. The pH values of the dough were 5.5, 5.2, 5.0, and 4.9, at fermentation times of 7 minutes, 1, 2, and 3 hours, respectively.

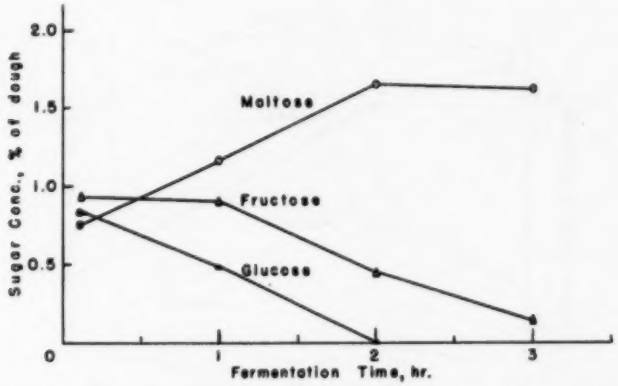

Fig. 2. Changes in sugar concentration during fermentation at 30°C. of a basic straight dough made with 3.0% yeast and 2.5% sucrose (equivalent to 1.4% of the dough).

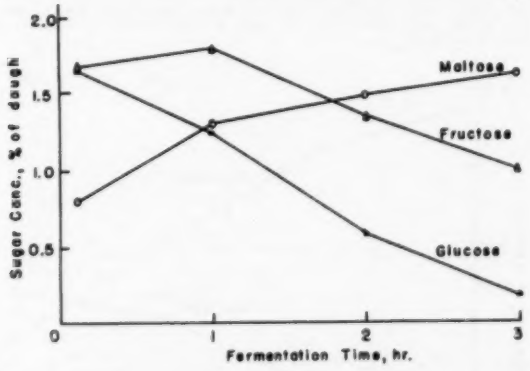

Fig. 3. Changes in sugar concentration during fermentation at 30°C. of a basic straight dough made with 3.0% yeast and 5.0% sucrose (equivalent to 2.94% of the dough). The pH values of the dough were 5.5, 5.1, 4.9, and 4.8 at fermentation times of 7 minutes, 1, 2, and 3 hours, respectively.

The southwestern baker's patent flour used in preparing the experimental doughs (unless otherwise stated) had been previously analyzed by the technics used in these experiments and was found to contain 0.01% glucose, 0.02% fructose, 0.10% sucrose, 0.08% maltose, and two unidentified components [5] (10).

[5] The sugars in wheat flour whose R_F values (0.025 and 0.034 with butanol-ethanol-water; 0.20 and 0.030 with butanol-propionic acid-water) corresponded to raffinose and melibiose were further examined. (The R_F values recorded for these two unknown sugars, designated melibiose and raffinose in the previous paper (10), were inadvertently reversed by a typographical error.) Upon boiling with 0.01 N sulfuric acid for 30 minutes the sugar corresponding to melibiose gave rise to glucose and fructose, showing that it is not melibiose but an oligosaccharide, perhaps a trisaccharide composed of glucose and fructose; the sugar having the same R_F as raffinose was evidently identical with this sugar since it yielded upon hydrolysis glucose, fructose, and melibiose.

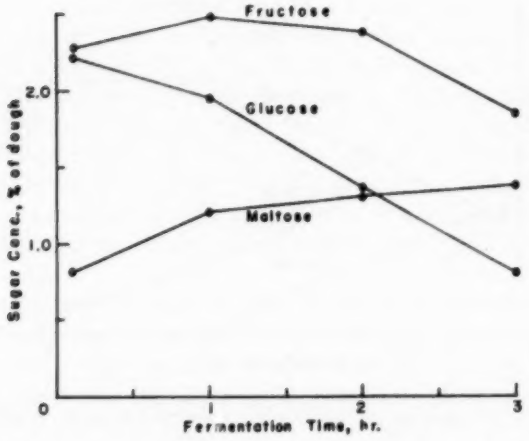

Fig. 4. Changes in sugar concentration during fermentation at 30°C. of a basic straight dough made with 3.0% yeast and 7.5% sucrose (equivalent to 4.35% of the dough). The pH values of the dough were 5.6, 5.2, 4.9, and 4.8 at fermentation times of 7 minutes, 1, 2, and 3 hours, respectively.

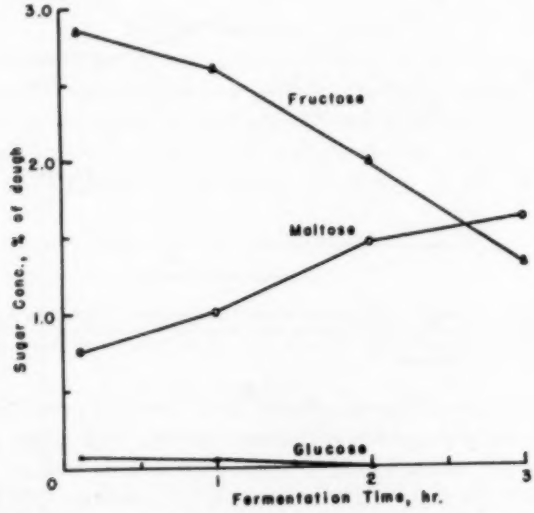

Fig. 5. Changes in sugar concentration during fermentation at 30°C. of a basic straight dough made with 3.0% yeast and 5.0% fructose (equivalent to 2.94% of the dough).

The first determinations of sugars were made in the doughs as soon as possible after mixing; about 7 minutes elapsed between the start of mixing and the inactivation of the enzymes. The basic doughs contained approximately 43% moisture. In the dough to which no

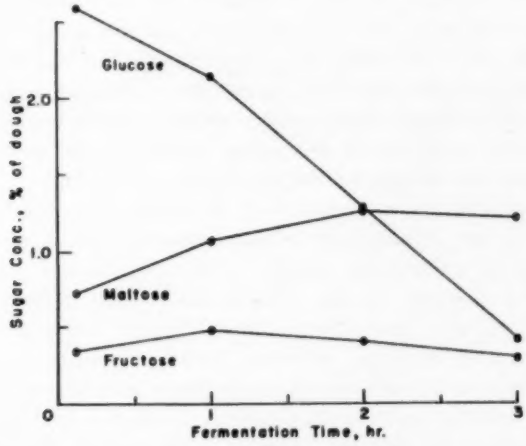

Fig. 6. Changes in sugar concentration during fermentation at 30°C. of a basic straight dough made with 3.0% yeast and 5.0% glucose (equivalent to 2.94% of the dough).

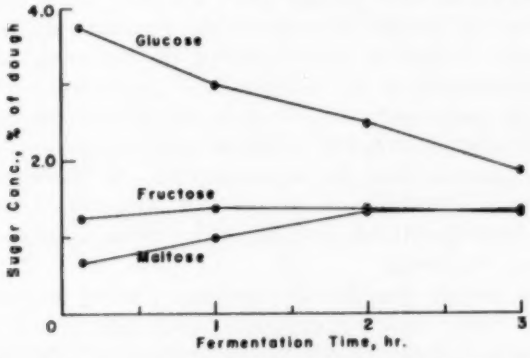

Fig. 7. Changes in sugar concentration during fermentation at 30°C. of a basic straight dough made with 3.0% yeast and 4.0% glucose and 4.0% sucrose, equivalent to 2.31% of each sugar in the dough.

sugar was added, the initial concentrations of glucose and fructose were 0.10 and 0.28% (Fig. 1). The higher value for fructose, as compared with glucose, is probably due to the hydrolysis of levosinelike oligosaccharides (glucofructosans) by invertase. The "initial" maltose concentration of the doughs to which no sugar and sugars other than maltose were added averaged about 0.8%, a relatively high value compared to the amount present in the flour.

The results for doughs which contained added sucrose showed that, even at the highest level (7.5% flour basis), it was completely hydrolyzed by the time the enzymes in the initial sample were inacti-

vated. The combined "initial" concentrations of glucose and fructose in doughs containing 2.5, 5.0, and 7.5% sucrose (flour basis), were found to be 1.72, 3.35, and 4.48% respectively. The theoretical values corresponding to the respective quantities of sucrose would be 1.56, 3.08, and 4.56%. These initial values, as well as those for other doughs, show excellent recovery of the sugars added in the dough formula and establish the validity of the analytical procedures.

In all doughs, the concentration of glucose decreased at a rapid and uniform rate throughout the fermentation period (Figs. 1 to 7). The change in the fructose content of the doughs was dependent on their glucose content. In the doughs made with different levels of added sucrose, the time which elapsed before a rapid decrease in fructose content occurred increased with increasing levels of glucose and fructose which arose from the hydrolysis of sucrose (Figs. 2, 3,4). These results indicate that glucose exerted a sparing action on the fermentation of fructose. This is confirmed by the experiments on doughs made with added glucose (Fig. 6), and with glucose plus sucrose (Fig. 7). In both doughs there was very little change in the concentration of fructose throughout the fermentation period. The slight increase in fructose content during the first hour of fermentation may be ascribed to the hydrolysis of levosinelike carbohydrates. In the dough made with 5.0% fructose, the glucose content was very low and the yeast was capable of fermenting fructose at a rather uniform and rapid rate from the beginning (Fig. 5). These results show that by proper adjustment of glucose and sucrose in the baking formula, fermentation can be accomplished without appreciable loss of fructose from the dough.

In these straight doughs, the maltose content increased during fermentation when there was sufficient glucose and/or fructose to support the fermentation (Figs. 2 to 5). However, in the dough made without any added sugars, the glucose and fructose were almost completely fermented at the end of 1 hour and the maltose level decreased upon further fermentation (Fig. 1). The sugar analyses in Table I for doughs to which corn sirups were added show trends in maltose concentration similar to those already discussed. The maltose content of fermenting doughs is a resultant of maltose production by amylase action and its removal by fermentation. The greater increases in maltose content of doughs when glucose or fructose is present in appreciable amounts is indicative that these hexoses are fermented in preference to maltose.

The sugar levels found in dough made by the commercial-type straight dough formula are given in Table II. The values for glucose,

TABLE I

SUGARS IN FERMENTING DOUGHS MADE WITH VARYING PERCENTAGES OF DIFFERENT CORN SIRUPS IN THE BASIC STRAIGHT DOUGH FORMULA (Yeast 3.0%, Flour Basis)

Fermentation Time	Sugar Content of Dough for Indicated Addition of Sirup Solids[1]			
	5.0% No. 1	5.0% No. 2	7.5% No. 2	5.4% No. 3
	Glucose			
7 minutes	0.60	0.62	1.00	1.19
1 hour	0.19	0.36	0.73	0.84
2 hours	0.00	0.00	0.29	0.31
3 hours	0.00	0.00	0.09	0.00
	Fructose			
7 minutes	0.24	0.25	0.24	0.33
1 hour	0.26	0.32	0.35	0.49
2 hours	0.00	0.08	0.29	0.30
3 hours	0.00	0.00	0.16	0.09
	Maltose			
7 minutes	1.38	1.53	2.38	1.30
1 hour	1.88	2.06	2.75	1.85
2 hours	2.08	2.41	3.16	1.99
3 hours	1.68	2.00	3.18	2.04
	Malto-Triose[2]			
7 minutes	0.40	0.36	0.56	0.46
1 hour	0.34	0.24	0.70	0.44
2 hours	0.14	0.15	0.49	0.32
3 hours	0.00	0.08	0.23	0.22

[1] Quantities added are expressed as percent of flour; 5.0 and 7.5% flour basis are equivalent to 2.94 and 4.35% of the dough weight, respectively.
Sugar concentrations are calculated on a crumb moisture basis equal to that of the dough. Dough and bread moisture contents were approximately 43.0 and 37.0%, respectively.
[2] Malto-triose concentration was obtained by converting absorbance readings to sugar concentration by reference to a maltose standard curve.

fructose, and maltose correspond closely with those obtained by the basic formula containing the same percentage of sucrose (5%, flour basis). Baker's yeast is incapable of fermenting lactose and the concentration of this sugar, which was present through the inclusion of nonfat milk solids in the formula, remained essentially unchanged.

Sponge and Dough Fermentation. The changes in sugar concentration in a fermenting sponge and dough made in the laboratory are shown in Fig. 8. The results obtained on sponges and doughs obtained from a commercial bakery are given in Fig. 9. In the sponge, which does not contain added sugars, the maltose concentration at first in-

TABLE II

SUGARS IN FERMENTING DOUGH MADE WITH A COMMERCIAL TYPE STRAIGHT DOUGH FORMULA CONTAINING 5% SUCROSE [1]

Fermentation Time	Sugar Content of Dough			
	Glucose	Fructose	Maltose	Lactose
	%	%	%	%
7 minutes	1.57	1.59	0.78	0.86
1 hour	1.09	1.48	1.08	0.80
2 hours	0.59	1.30	1.32	0.80
3 hours	0.20	0.82	1.48	0.89

[1] The dough was made with 3% yeast; original sucrose content expressed on dough weight was 2.8%.

creased and then decreased rapidly during the later stages when the glucose and fructose became depleted. With the addition of sugars (sucrose in the case of Fig. 8, and glucose and sucrose in Fig. 9), at the dough stage, they are preferentially fermented and there is an initial increase in maltose content, as a result of amylase activity. Soon, however, the rate of fermentation of maltose exceeds its rate of production by amylase action and the concentration decreases gradually. A comparison of the maltose curves in Figs. 8 and 9 with those obtained with straight doughs supports the view that during the time yeast fermentation in the sponge was dependent on maltose, the yeast developed a greater ability to ferment this sugar. This resulted in a greater fermentation of maltose in the dough stage than occurred in the straight doughs. As a result of the greater use of maltose in both

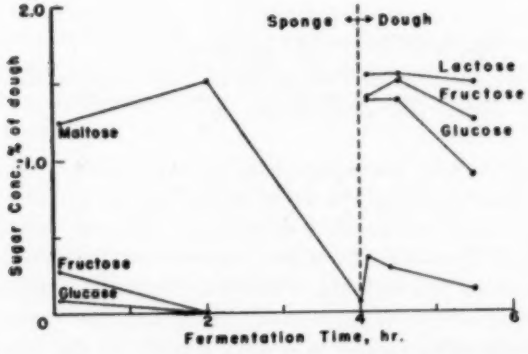

Fig. 8. Changes in sugar concentration in a sponge and dough, each fermenting at 30°C. A commercial-type formula was used with 2.0% yeast and 5.0% sucrose, flour basis. The sucrose was added at the dough stage and was equivalent to 2.74% of the total dough weight. The pH values for the sponge were 5.4, 5.2, 5.0, and 4.7 at 7 minutes, 1, 2, and 3 hours respectively. The pH values determined on the dough after 7, 30, and 90 minutes of fermentation were 5.3, 5.1, and 5.0, respectively.

the sponge and dough stage, the maltose content of the doughs at the end of 3 hours' fermentation is much lower than that of the straight doughs. The curves in Fig. 8 for the dough stage again show that glucose is fermented initially at a more rapid rate than fructose and that the amount of lactose remains essentially unchanged. In the commercial bakery, the formula contained 3.0% each of glucose and sucrose; in this case, the fructose concentration changed only slightly while the glucose content fell rapidly (Fig. 9). Lactose was present in the commercial dough but was not determined.

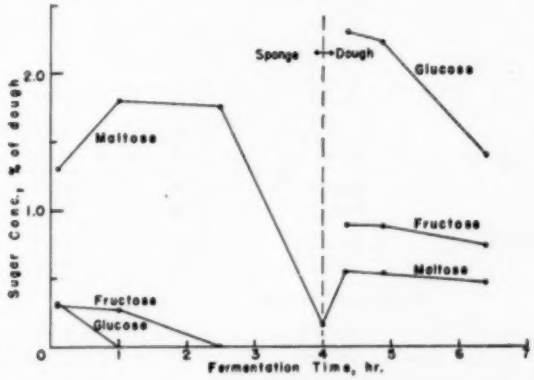

Fig. 9. Changes in sugar concentration in samples of a fermenting sponge and dough taken from a commercial bakery. The formula contained 3.0% of glucose and 3.0% of sucrose (total flour basis), equivalent to 1.65% of each sugar in the dough. Sponge was fermented 4.0 hours maintained at 27°C. (80°F.); dough was fermented a total of 35 minutes at 27°C. and proofed about 70 minutes at 40.5°C. (105°F.). Lactose was present but not determined.

Residual Sugars in Bread. In several cases, the doughs were baked and the sugar content of the bread crumb determined. The results are given in Table III together with the concentration of the different sugars in the doughs at the end of the fermentation period. The sugar contents of the doughs and corresponding breads were very similar, but the bread contained slightly lower percentages of glucose and fructose.

It is noteworthy that the bread prepared by the sponge and dough procedure which contained 3.0% glucose and 3.0% sucrose in the baking formula contained only 0.7% maltose as compared with 1.5 to 2.1% for breads made by the straight dough procedure with 5% additions of sucrose, fructose, or corn sirup. In fact, the maltose content of the bread made by the sponge and dough method is essentially the same as that of bread made by a straight dough procedure in which no sugars were added.

TABLE III

COMPARATIVE SUGAR CONTENTS OF BREAD AND OF DOUGHS AT THE END OF THE PROOF PERIOD

Sugar in Formula (Flour Basis)	Procedure	Sugar Content[1]		
		Glucose	Fructose	Maltose
		%	%	%
	Straight Dough			
None	Dough	0.00	0.00	0.58
	Bread	0.00	0.00	0.58
Sucrose, 2.5%	Dough	0.00	0.44	1.61
	Bread	0.00	0.14	...
Sucrose, 5.0%	Dough	0.18	1.00	1.62
	Bread	0.14	0.79	1.50
Glucose, 5.0%	Dough	0.42	0.30	1.22
	Bread[2]	0.23	0.13	1.21
Fructose, 5.0%	Dough	0.00	1.32	1.61
	Bread	0.00	1.11	1.68
Corn Sirup No. 1, 5%	Dough	0.00	0.00	2.00
	Bread	0.00	0.00	2.10
	Sponge and Dough			
Glucose, 3.0% and Sucrose, 3.0	Dough	1.40	0.74	0.47
	Bread	1.10	0.66	0.69

[1] Sugar concentrations are all expressed on a moisture basis of 43.0%. Moisture contents of the doughs and bread were approximately 43.0% and 37.0%, respectively.
[2] These results were obtained on bread made at a later date than the dough. The glucose, fructose, and maltose contents of the dough from which this bread was prepared were 0.33, 0.18, and 1.34%, respectively, at the end of the proof period.

Yeast Fermentation in a Synthetic Nutrient Solution Containing Different Sugars. Changes in sugar concentration and gas production rates were determined with synthetic nutrient solutions containing respectively sucrose, maltose, sucrose plus maltose, and maltose followed by additions of glucose (or glucose and maltose) after a period of fermentation. The results are presented in Figs. 10 to 13 inclusive.

The changes in sugar concentration follow similar patterns to those observed in bread doughs. The great rapidity with which sucrose is hydrolyzed is shown in Fig. 10: 2 minutes after the addition of the yeast, more than 90% of the sucrose had been inverted. Initially the glucose was fermented more rapidly than fructose but after about 1 hour the concentration of the two sugars decreased at similar rates. In other experiments, where glucose and fructose were employed in separate nutrient solutions, their rates of fermentation and the production of carbon dioxide were very similar. The yeast was, therefore,

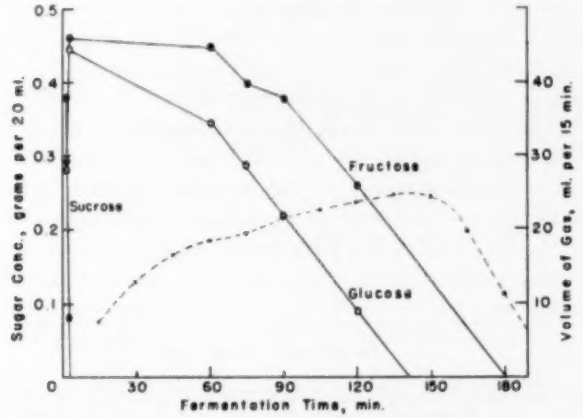

Fig. 10. Changes in sugar content of a synthetic nutrient solution undergoing fermentation at 30°C. The nutrient solution contained 0.6 g. of yeast and 1.0 g. of sucrose per 20 ml. The rate of gas production is shown by a broken line.

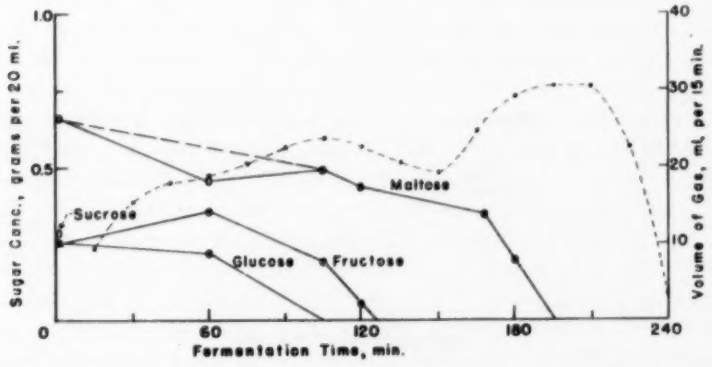

Fig. 11. Changes in sugar content of a synthetic nutrient solution undergoing fermentation at 30°C. The nutrient solution contained 0.6 g. of yeast, 0.75 g. of sucrose, and 0.75 g. of maltose monohydrate per 20 ml. The rate of gas production is shown by a broken line.

capable of fermenting fructose at a rapid rate when no glucose was available.

The results of an experiment in which sucrose and maltose were both present in the nutrient solution from the beginning of fermentation are illustrated in Fig. 11. The glucose arising from sucrose inversion was fermented more rapidly than the fructose; moreover, the maltose was fermented at a slow rate until the glucose and fructose were exhausted. There was a short period occurring between the exhaustion of the hexoses and the increase in the rate of maltose fer-

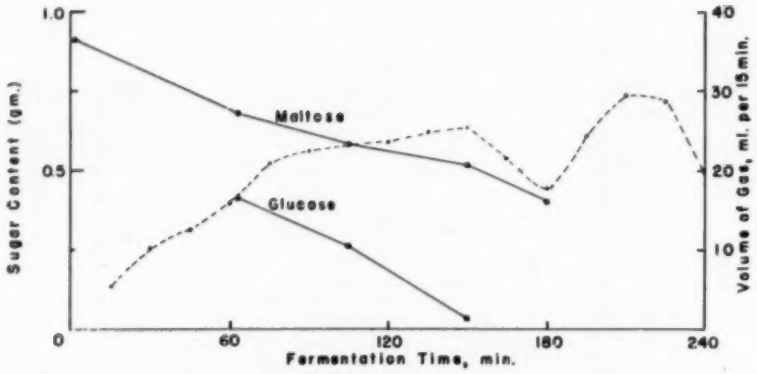

Fig. 12. Changes in sugar content of a synthetic nutrient solution undergoing fermentation at 30°C. The original solution contained 0.6 g. of yeast and 1.0 g. of maltose monohydrate per 20 ml. After 60 minutes of fermentation, 0.5 g. of glucose was added. The rate of gas production is shown by a broken line.

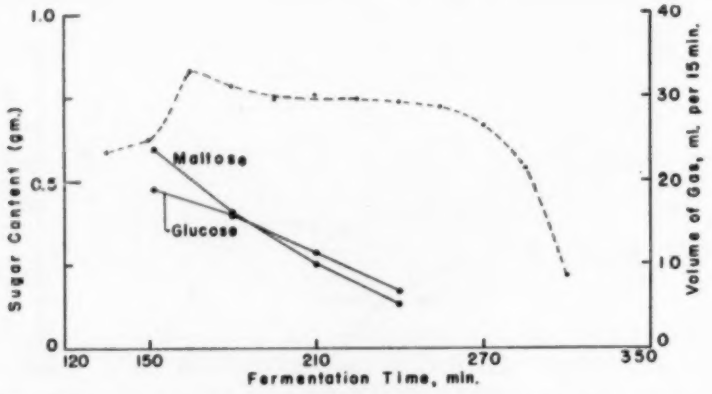

Fig. 13. Changes in sugar content of a synthetic nutrient solution undergoing fermentation at 30°C. The original solution contained 0.6 g. of yeast and 1.0 g. of maltose monohydrate per 20 ml. After 150 minutes of fermentation, 0.5 g. of glucose and 0.5 g. of maltose monohydrate were added. The rate of gas production is shown by a broken line.

mentation when the rate of gas production dropped appreciably. These results support the view that the temporary decrease in the rate of gas production observed in fermenting bread doughs (13, 16, 26) may be attributed to a change-over from the fermentation of glucose and fructose to maltose. In Fig. 11 there is some indication of a smaller and earlier inflection in the gas production curve than that ascribable to the initiation of maltose fermentation; this occurred at the time the yeast began to ferment fructose more rapidly.

Experiments were conducted in which maltose was the only sugar

originally present in the nutrient solution; after 60 minutes and 150 minutes respectively, glucose was added. The curves in Fig. 12 show that 60 minutes is too short a time for the yeast to become completely adapted to the fermentation of maltose. The addition of glucose resulted in an increase in the rate of gas production and a slight decrease in maltose fermentation until the glucose disappeared; upon the exhaustion of glucose there was a sharp temporary decrease in gas production. However, when maltose fermentation was allowed to proceed for 150 minutes before glucose and additional maltose were added, no temporary decrease in gas production occurred. After the addition of the glucose, the maltose concentration continued to decrease at a rapid rate, a rate which was somewhat greater than that for glucose.

These results provide an explanation for the marked difference in the maltose content of the straight dough made with 5.0% sucrose (Fig. 3) and the corresponding dough, with the same content of flour and sucrose, made by a sponge and dough procedure (Fig. 8). In the straight dough, the maltose content progressively increased from an initial value of 0.78% to 1.46% after 3 hours' fermentation. In contrast, the maltose content of the sponge decreased from 1.25% after mixing to 0.08% after 4 hours' fermentation; in the dough stage the maltose content decreased from 0.37% immediately after mixing to 0.16% after 1.5 hours' fermentation. In the sponge and dough method, the sugar is added at the dough stage; the hexoses in the flour and those produced by invertase action soon become exhausted and fermentation is then supported by the maltose produced by amylase action. By the time the sucrose is added at the dough stage, the yeast has become fully adapted and continues to ferment maltose at a rate which is quite comparable to the rates of fermentation of the glucose (and fructose) produced by sucrose inversion. In the straight dough process, the sucrose is present from the beginning and the glucose and fructose are preferentially fermented to maltose. As a result, the yeast did not become "adapted" and the rate of maltose fermentation was less than its rate of production.

Acknowledgment

The authors are indebted to the Corn Products Sales Co., New York, N.Y., for a research grant in support of this investigation.

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EVALUATING SEMOLINA COLOR WITH PHOTOELECTRIC REFLECTOMETERS [1]

S. A. MATZ AND R. A. LARSEN

ABSTRACT

The reflectance spectra of a series of semolina samples of different colors were determined by means of the Rapid-scanning Spectrophotometer. Comparison of these spectra with visual evaluations of the samples indicated that percent reflectance in the region of 435 $m\mu$ can be used as a single-figure score denoting color-quality.

Several other reflectometers were investigated to determine their utility for estimating color-quality. Results obtained using the Hunter Color-Difference Meter, the Photovolt Reflection Meter and the Densichron (listed in the order of decreasing precision) indicate that these instruments give useful estimates of semolina color when filters transmitting in the region of the visible spectrum near 435 $m\mu$ are employed.

The color of macaroni products is usually considered to be the most important criterion of their quality (39). Macaroni color can be influenced by many factors, including particle size and enzyme activity of the semolina (16), mixing conditions (17) and pressing conditions (36). Among its chief determinants are the pigments present in the semolina from which it is made. The color-quality of semolina is primarily a function of the variety of wheat; Mindum, for example, consistently gives a semolina of better color than that milled from Pentad (12). Normally, however, the paste products manufacturer does not know which variety of wheat the miller is using, so he must rely upon a visual examination of the semolina to determine its suitability for his purposes. As a result, the more highly colored semolinas command a premium on the market.

An objective and dependable method for measuring the color of semolina would be of obvious value, and numerous attempts have been made to devise such a procedure. Many macaroni manufacturers are accustomed to grading semolina color visually, comparing the unknown sample with a standard. Some durum millers furnish their customers with printed color charts and refer to these standards when making quotations. This simple method has some value, but its many shortcomings are immediately apparent. For example, no printed color can satisfactorily duplicate the appearance of a semolina sample; paper standards are particularly susceptible to fading and soiling.

[1] Manuscript received July 8, 1953. This paper reports research undertaken at the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 436 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

and the conditions of illuminating and viewing are apt to be different every time the charts are used.

Since it is much easier to measure the transmitted color of a transparent sample than it is to measure the reflected color of an opaque substance, many investigators have tried to simplify the problem of color evaluation by extracting flour or semolina pigments and working with the clear solutions. (1, 6, 7, 8, 10, 15, 25, 26, 30, 32, 40).

Although colorimetric or spectrophotometric measurements of extracted pigments add some degree of objectivity to color evaluations, they have, of necessity, many serious defects when used for determining semolina color. In the first place, pigments are not the sole determinants of color in granular substances such as semolina (16, 17, 36). Particle size and index of refraction, among other factors, affect color (20). Thus, it is extremely difficult – from an optical standpoint – to predict or classify the reflected color of a granular translucent substance from a characterization of the transmissivity of its pigments alone. Furthermore, granule size, shape, and permeability will affect the rate of extraction of pigments, and it remains to be proved that any of the extraction methods which have been suggested removes all of the pigments which significantly affect product color. It is known that complete recovery, even of the carotenoid pigments, is difficult, especially when particle size is relatively large.

A logical way to avoid the disadvantages of the extraction procedures is to measure the color of the product itself. An early method for comparing the color of cereal products was the well known Pekar test, which can be used with semolina as well as with flour. The accuracy of this test is entirely dependent on the skill and visual acuity of the individual operator. Jago and Jago (19) attempted to overcome the disadvantages of the Pekar test by using a modified Lovibond tintometer to measure the color of flour slicks. The tintometer uses standardized colored glass slides to duplicate the color of a sample. It has never become popular for grading flour or semolina. Some of the difficulties attending its use in other fields were pointed out by Wesson (38) who found considerable disagreement among tintometer values reported in a collaborative study of vegetable oil samples. Many of the objections which Wesson reported would also affect use of the instrument with semolina.

Other early attempts to establish an objective colorimetric method for measuring the color of cereal products adapted the method used by Nickerson (33) to grade hay and cotton. Essentially, this procedure consists of matching the commodity color with combinations of Mun-

sell papers (31) under standard conditions of viewing and illumination. The papers are usually formed into Maxwell discs and rapidly rotated to blend the colors. In some cases, the commodity sample is also rotated to give a uniform field. When the standard papers have been adjusted to match the unknown color, the percentage of each standard color in the field is determined by measuring along the perimeter of the discs. The color is then characterized by a notation combining the percentage of each color and its Munsell code identification. Grewe, Marshall, and Harrel (11) used this method to get a good quantitative measurement of bread color. Fifield, Smith, and Hayes (9) applied colorimetry to durum products using the N-A disc colorimeter described by Baker, Parker, and Freese (2) which measures sample color in terms of the percentage of standard red, yellow, black, and white required to match it.

Colorimetric methods such as those described above have many advantages. Specimen color is measured directly, minimizing sample preparation and the errors that accompany it, cost of equipment is usually moderate, standards are easily reproduced and can often be directly related to commercial grading systems, and the test is nondestructive. However, problems arise from the nonuniformity of ocular response in different observers and from difficulty in maintaining constant viewing and illuminating conditions.

In recent years, electronic devices which can measure the reflected colors of substances have become commercially available. These reflectance measuring spectrophotometers eliminate many of the problems associated with methods requiring matching of fields by the human eye. The viewing and illuminating systems are integral parts of the apparatus and so are much less susceptible to chance alterations than are systems separate from the measuring device. They are capable of giving values which can be duplicated with a high degree of precision by different operators. As a result of these considerations, it seems reasonable to assume that these devices could be adapted to color-grading of semolina. To establish their utility for this purpose a survey of several photoelectric reflectometers was undertaken. The correspondence between semolina rankings according to reflectance values as determined by the Rapid-scanning Spectrophotometer, the Photovolt Reflection Meter, the Densichron, the Hunter Color-difference Meter and the Color-Eye and a visual ranking of the same samples was used as an indication of the relative merit of each apparatus. A statistical analysis of the reflectance data secured through use of the Photovolt, Densichron, and Hunter devices was made in order to determine their relative precision.

Materials and Methods

The Rapid-scanning Spectrophotometer used in this study employs an oscilloscope to record the percent reflectance throughout the range 400 $m\mu$ to 700 $m\mu$. A set of coordinates is etched on the face of the cathode-ray tube and the curve may be photographed to provide a permanent record. The sample is viewed at an angle of 0° (normal to the sample surface) and illuminated at a 45° angle.

The Densichron is essentially a photoelectric device designed to measure film density but it can be converted, through use of a reflection head and filter holders, to function as a colorimetric reflectometer. Illumination is at a 45° angle and the viewing angle is 0°. The blue filter used in this study was the Wratten No. 48 transmitting from 380 to 520 $m\mu$ with a peak at 466.5 $m\mu$ (5) and the blue sensitive photocell viewing unit has peak sensitivity at about 420 $m\mu$. The Magnephot system developed by Kalmus and Striker (22) is employed in this device to provide an A.C. output from the photocell and permit amplification by conventional A.C. means. Advantages of this method are greater sensitivity and reduced zero drift.

The Photovolt reflection-meter has a search unit which incorporates ring-shaped barrier-layer photocells so arranged that only diffuse reflection is measured. Illumination is at 0° and viewing is within a range of several degrees averaging about 45°. Filters are interposed in the illuminating beam (35). The blue filter used with this instrument has peak transmission at 420 $m\mu$ and spectral width of about 60 $m\mu$ (28). The Photovolt reflection meter has been used with considerable success for measuring the relative color of parboiled rice (23).

Some cursory experiments were carried out with the tri-stimulus color matcher described by Bentley (3) and the Hunter color difference meter (14) which has given good results with many agricultural products such as tomato purees (41, 42). The Kent-Jones and Martin Flour Colour Grader (24, 27), which provides a value related to reflectance in the region near 530 $m\mu$ was investigated.

Various working standards, each calibrated against magnesium oxide surfaces, were used in this study. Enameled or ceramic plates were found to be the most convenient working standards and were entirely reliable as long as the reflecting surfaces were kept clean and free from scratches or abrasions. No changes in calibration due to aging were noted during the period of this study. A series of neutral gray plastic color chips supplied by the Container Corporation of America (18) was used in some instances, but these standards presented some practical difficulties due to the curvature and softness

of the reflecting surfaces. The Munsell papers were considered to be too susceptible to soiling to be satisfactory as standards in a study of this kind, and they were not used.

Cylindrical glass cuvettes with optically clear bottoms were used as sample containers except with the Color-Eye and Kent-Jones devices, in which cases the special cuvettes provided by the manufacturer were used. Cuvettes were filled to a depth sufficient to extinguish the transmitted light.

A series of 11 samples of durum semolinas and four samples of durum flours were obtained from commercial sources. The semolinas represented a fairly wide range of color for this type of commodity but no extremely poor or unmarketable samples were included. It was felt that samples grossly aberrant in color could be adequately detected by visual inspection. The samples are characterized by the data for protein, ash, visual appearance and granulation given in Table I.

TABLE I

PHYSICAL AND CHEMICAL CHARACTERISTICS OF SEMOLINAS AND DURUM FLOURS

Sample Number	Protein[1] (N × 5.7)	Ash[1]	Visual Rank	Sieve test; overs %			
				20W	40W	60W	100W
			Semolinas				
	%	%					
1	10.8	0.63	1–2	0	24	55	17
2	10.6	0.45	5	0	25	53	18
3	11.0	0.71	7	0	21	50	17
4	10.6	0.66	3	0	24	50	19
5	10.6	0.75	6	0	21	53	20
6	10.6	0.64	4	0	24	50	19
7	10.9	0.60	8	0	29	38	18
8	11.0	0.70	10	0	20	46	16
9	11.0	0.62	9	0	25	54	14
10	10.7	0.56	1–2	0	28	48	19
11	12.2	0.65	11	0	20	51	18
			Flours				
12	11.2	0.59	15	0	0	Trace	17
13	11.5	0.77	14	0	Trace	0.5	1.0
14	11.8	0.75	13	0	Trace	1.0	0.5
15	12.8	0.84	12	0	0.5	0.5	0.5

[1] 14% moisture basis.

In the column of Table I marked "Visual Rank" is given the rank of each sample in the series according to a visual estimate by the authors of its suitability for macaroni manufacturing purposes. Number one indicates the sample of best color and No. 15 indicates the worst sample. These ratings were arrived at by comparing the samples

side by side under uniform conditions and represent approximately the degree of yellowness of the sample.

Consideration was given to eliminating the effect of particle size on reflected color by such expedients as sieving, pasting, or compression. However, this approach was soon abandoned since the object of this study was to measure electronically the color of semolina as the eye of the user sees it. In visual examination, both pigmentation and granulation are important.

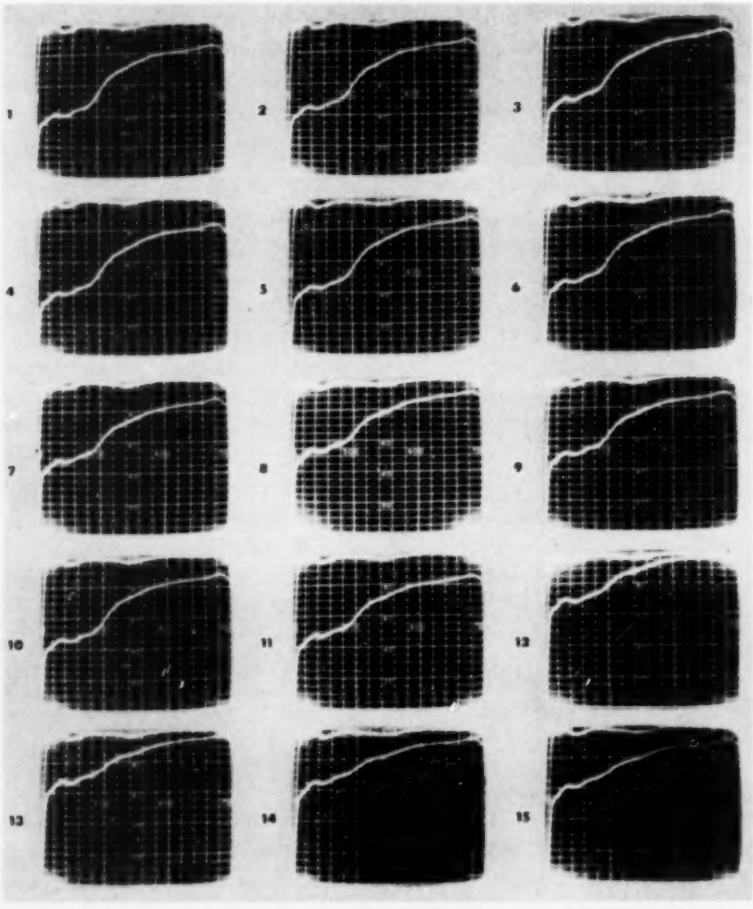

Fig. 1. Reflectances of semolina samples as determined by the Rapid-scanning Spectrophotometer. Percent reflectance on the vertical axis and mu wavelength on the horizontal axis.

Results

Rapid-scanning Spectrophotometer Data. Typical reflectance spectra of the 15 durum product samples obtained by the rapid-scanning spectrophotometer are reproduced in Fig. 1. Repeat readings were made of all samples, and, in some cases, as many as five readings were made. The maximum difference in replicate values was 2% reflectance. Reference to Fig. 1 indicates that the greatest variation between the different spectra occurs in the region of 410 mμ to 500 mμ. The outstanding feature in this area is the peak at about 435 mμ. The percent reflectance at 435 mμ in all of the samples is recorded in Table II.

TABLE II

REFLECTANCE OF DURUM PRODUCTS AT 435 Mμ DETERMINED BY THE RAPID-SCANNING SPECTROPHOTOMETER

Sample Number	Reflectance		Visual Evaluation
	Mean	Rank	
	%		
1	42	2	1–2
2	45	4–5	5
3	48	7–8–9	7
4	40	1	3
5	44	3	6
6	45	4–5	4
7	48	7–8–9	8
8	53	11	10
9	50	10	9
10	46	6	1–2
11	48	7–8–9	11
12	70	15	15
13	68	14	14
14	65	13	13
15	61	12	12

There is a spread of 13% between extreme values of the semolina group, a spread of 9% between extremes of the flours, and a spread of 30% for the entire series. Readings on different portions of the same semolina never varied more than 2% and the average difference between duplicates was 1%. Numbers in the column headed "Rank" denote the relative position in the group of the samples rated in an inverse relation to the reflectance value – an arbitrarily chosen arrangement. Comparison of these numbers with the visual rank shows a close correspondence. The greatest differences between visual and spectrophotometer ratings occur in the case of sample 10, considered to be a tie for first in the visual evaluation series. The rank-order correlation coefficient (ρ) calculated from the visual ranks and the spec-

trophotometer ranks for the semolinas is +0.79 (34), significant at the 1% level.

Densichron Data. Table III summarizes the data obtained using the Densichron equipped with a filter transmitting in the sensitive range previously described, near 435 mμ.

TABLE III

REFLECTANCE OF DURUM PRODUCTS DETERMINED BY THE DENSICHRON

Sample Number	Number of Determinations	Reflectance: Blue Filter				Visual Rank
		Mean	Range	Standard Deviation	Rank	
		%	%	%		
1	68	38.2	5.4	1.3	2	1–2
2	38	40.5	5.4	1.1	6	5
3	26	42.9	4.4	1.7	9	7
4	26	37.1	5.0	2.2	1	3
5	68	40.1	5.2	1.2	5	6
6	38	38.8	4.5	1.1	3	4
7	38	42.2	3.5	0.8	8	8
8	38	46.4	4.2	1.3	11	10
9	38	43.6	4.3	1.3	10	9
10	38	40.0	4.8	1.8	4	1–2
11	38	40.9	4.9	1.5	7	11
12	8	55.5	1.3	0.4	15	15
13	8	54.6	1.1	0.4	14	14
14	8	53.6	0.4	0.1	13	13
15	8	49.8	0.6	0.2	12	12

Reference to Table III indicates that the difference between extreme reflectance values for the semolina samples was 9.3%, for the flours, 5.7% and for the entire series, 18.4%. All of these figures are smaller than the corresponding ones for the Spectrophotometer series. This result was expected, since the Spectrophotometer readings were taken at the wave length of maximum sensitivity, while the filter used in the Densichron isolates a fairly wide band of the spectrum including areas of less sensitivity. A rather large variation between replicate readings of each sample is shown in Table III. This is, undoubtedly, due in large part to the considerable number of readings which were made on many different occasions over a period of several months, but it is also an indication of the extent of variations due to experimental error that can be expected when this device is used.

Reference to the sixth and seventh columns of Table III indicates that sample rank as determined by its reflectance is closely related to its visual rank. Statistically, the ρ is 0.84 for the first eleven samples, significant at the 1% level. No sample is separated more than four places from its position as determined visually.

Photovolt Data. Table IV summarizes the data obtained using the Photovolt apparatus equipped with a blue filter. In this and subsequent tables, data for the first six samples only will be presented, since they seem to provide an adequate basis for comparison with previous work.

TABLE IV

REFLECTANCE OF SEMOLINA DETERMINED BY PHOTOVOLT REFLECTION METER EQUIPPED WITH BLUE FILTER

Sample Number	Reflectance				Visual Rank
	Mean[1]	Range	Standard Deviation	Rank	
	%	%	%		
1	47.3	2.5	0.8	2	1
2	49.1	0.7	0.2	5	4
3	51.5	3.3	1.0	6	6
4	46.9	2.1	0.6	1	2
5	48.9	1.9	0.6	3	5
6	49.0	2.2	0.7	4	3

[1] Of nine determinations.

The results approximate those obtained by using the Densichron. Photovolt reflectance readings are higher throughout than the Densichron values, as a result of the slightly different spectrum ranges isolated by the filter used with this instrument. However, the samples are rated in substantially the same order. Statistically, the $\rho = 0.77$ for these six semolinas. The spread between the highest and the lowest values is somewhat less in Table IV than in Table III, i.e., 4.6% vs. 5.8%. The differences between replicates are considerably less in the Photovolt series, but the number of determinations was also much less. In Fig. 2, the Densichron and Photovolt reflectance values which

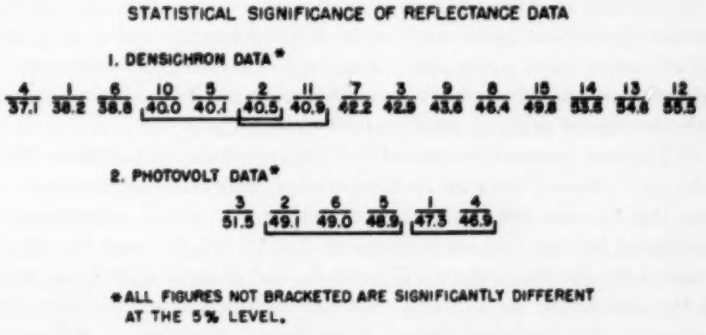

Fig. 2. Statistical significance of reflectance data.

are not significantly different in a 5% level multiple comparisons test are bracketed (4). In other words, the probability is greater than 1 in 20 that the difference between the bracketed figures could occur as a result of chance variation. All other values are significantly different at this level. (In the Densichron series, it is recognized that the variances 1 to 11 and 12 to 15 are homogeneous, respectively, but that across these groups they are heterogeneous. Therefore, the standard error of a treatment mean is based on the 11 measurements. Inspection suffices to separate groups 12 through 15.)

Hunter-Color-Difference Meter Data. Table V summarizes the data obtained using the Hunter color difference meter. The figures listed are not directly related to percent reflectance in any narrow region of the spectrum. The "a" values indicate greenness when they are minus, as they are in this case; the "b" values indicate yellowness when plus, and the R_d values indicate grayness.

TABLE V

HUNTER COLOR-DIFFERENCE METER VALUES FOR SEMOLINAS

Sample Number	R_d			a			b			b Rank	Visual Rank
	Mean[1]	Range	Standard Deviation	Mean[1]	Range	Standard Deviation	Mean[1]	Range	Standard Deviation		
1	66.4	1.1	0.38	−1.5	0.2	0.09	22.3	0.2	0.08	2	1
2	66.8	0.4	0.17	−1.5	0.3	0.12	22.2	0.6	0.21	3	4
3	67.5	0.4	0.16	−1.1	0.2	0.09	20.3	0.0	0.00	6	6
4	64.9	0.8	0.27	−1.4	0.3	0.12	22.5	0.2	0.08	1	2
5	66.6	0.9	0.39	−1.4	0.1	0.06	21.7	0.3	0.10	5	5
6	66.4	0.6	0.23	−1.5	0.1	0.01	21.8	0.4	0.15	4	3

[1] Of five values.

Reference to Table V indicates that the spread between the highest and lowest values of each series is considerably less than that obtained using the previously described instruments. However, the sample rank based on the *b*-coordinate value correlates quite well with the visual rank, ρ being +0.89 in this case.

To secure a measurement of the ability of the Densichron, Photovolt, and Hunter devices to differentiate between the semolina samples, the F ratios for the variance between vs. within semolinas were calculated for the first six samples in Tables III, IV, and V (13). The F ratios for the Densichron, Photovolt, and Hunter devices were 65.9, 44.25, and 158.0, respectively. All are significant at the 0.1% level, indicating the machines have a high degree of ability to differentiate

between the semolinas. The within-sample mean squares (variances) can be used as an indication of the precision of each device. These values are 1.96, 0.54, and 0.02, respectively. Ratios of these variances provide a measure of the relative precision of the three machines; from these it appears that the Hunter apparatus is more accurate than the Photovolt, while the Photovolt is more accurate than the Densichron. A more rigorous and complex multiple comparison test can be avoided in this instance because one of the within-sample mean squares was greatly different from the other two.

"Color-Eye" Data. Table VI summarizes the data secured by using the Industrial Development Laboratories' Industrial Tristimulus Color Matcher, also called the "Color-Eye". Results obtained with all three of the instrument's filters are included in the table in order to illustrate significant characteristics of the apparatus.

TABLE VI

PERCENT REFLECTANCE OF SEMOLINA SAMPLES DETERMINED BY THE "COLOR-EYE"

Sample Number	Amber Filter		Green Filter		Blue Filter	
	Mean[1]	Range	Mean[1]	Range	Mean[1]	Range
1	85.6	0.5	80.2	0.6	53.2	1.4
2	86.4	1.1	80.2	1.6	54.1	4.0
3	86.7	1.1	81.3	1.4	54.2	3.8
4	85.5	1.0	80.1	1.4	53.5	3.0
5	84.3	0.6	78.7	0.9	51.6	1.9
6	86.5	1.2	81.3	1.8	56.6	5.6

[1] Of two determinations.

The Color-Eye does not give reflectance value readings for semolina which are adequately reproducible. For example, the range of readings for different determinations for each sample with the blue filter approaches the spread between the highest and lowest means of the series. This fluctuation is at least partly due to the small area of sample viewed – less than one-half inch. When relatively large sample areas are viewed, there is some tendency for the usual variations in color of different sections of the sample surface to "average" and give a representative value for the entire sample.

Flour Color Grader. A study of the characteristics of the Kent-Jones and Martin Flour Colour Grader led to the conclusion that this instrument would not be useful for evaluating semolina. This instrument employs a filter passing light in a region of the spectrum outside of the sensitive range previously described.

Discussion

In spite of the large amount of work that has been done on the color of semolina, it is interesting that no method of measuring this color has ever been widely adopted. A great deal of the trouble, no doubt, has arisen from the lack of a clear-cut differentiation between the use of transmitted light and reflected light for such work. Since semolina products are opaque rather than clear, the color observed by the eye is due to reflected light and hence, any optical method for measuring this color should be based upon reflectance principles.

If this premise is accepted, it is readily seen why the work done on measuring the color of light transmitted through a clear solution of the pigments extracted from semolina bears only an indefinite relationship to the color as seen by the eye. Mathematically, it is extremely difficult to relate reflected and transmitted color (20, 21, 28), and moreover, the measurement of transmitted color neglects such important factors as particle size.

In this regard, the question might be raised as to the relationship of the color of semolina to the color of macaroni or spaghetti made with that semolina. Is the importance of particle size to the color of semolina lost during processing? Is the pigment loss due to enzyme action during macaroni processing relatively constant for different batches of semolina? What effect do different processing methods have on color of the finished product? These questions remain to be answered.

Regardless, considering only the color of semolina, this study has shown that the spectral curves of different samples are essentially nonmetameric – that is, the reflectance curves are similar in shape and do not intersect. However, the quantity of light reflected at any one wavelength differs between semolina samples. Since the greatest differences in the amount of reflected light appear to occur near 435 $m\mu$, comparisons should be made near this wavelength.

Of the several colorimetric instruments investigated, the Densichron, the Hunter color-difference meter, and the Photovolt reflection meter seem to offer the most promise as control instruments. A Wratten No. 48 filter should be used with the former instrument, while the single-glass blue filter furnished by the manufacturer is suitable for use with the Photovolt. The *b*-coordinate of the Hunter apparatus should be used. The Rapid-scanning Spectrophotometer obviously has many advantages as a research instrument, but it is questionable whether such an elaborate device is needed for control work.

Acknowledgments

The authors gratefully acknowledge the assistance of Mr. Kenneth Wood and Mr. J. G. Haynes in making the statistical analysis of reflectance data, and of Mr. John McMullen and his group in performing the chemical determinations.

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DETERMINATION OF REDUCING SUGARS IN BREAD BY BIOLOGICAL METHODS[1]

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ABSTRACT

The quantities of hexoses (D-glucose and D-fructose) and of maltose in bread crumb may be determined without extraction by preparing a "dough" and measuring manometrically the respective quantities of carbon dioxide produced upon fermentation by baker's yeast and by a special type of brewer's yeast incapable of fermenting maltose. The pressure readings are converted into sugar values by means of appropriate factors obtained by adding known quantities of the respective sugars to the residue and measuring the gas production. Lactose may be determined by the usual gravimetric methods on the doughs from which the hexoses and maltose have been removed by fermentation.

Bread made by both the straight and sponge dough processes from a formula containing sucrose had a higher hexose content than that made by a formula employing a similar amount of dextrose hydrate. Bread made by the straight dough process had a higher content of maltose than bread made by the sponge dough process. The hexose content of 18 commercial breads varied from 0.0% to 3.54%, the maltose from 0.15% to 2.85%, and total sugar from 2.36% to 5.65%, based on crumb moisture.

Biological methods have been used by many observers to estimate the sugars in plant extracts, starch hydrolysates, etc. These methods depend on the selective fermentation of the various sugars by certain types of yeast, or on the control of the fermenting properties of yeast by varying the pH.

Schultz and Landis (8) used baker's yeast to study the rate of enzyme hydrolysis of soluble starch by a fermentation method. Schultz and Kirby (7) selectively fermented dextrose or levulose, sucrose, and maltose in the presence of each other by employing a pure culture of a *Mycoderma* which fermented dextrose or levulose but not sucrose or maltose, an invertase preparation, and baker's yeast. Schultz *et al.* (9) separated by selective fermentation the sugar components of starch hydrolysates. More recently Gates and Kneen (4) measured the maltose activity of various mold brans by utilizing the selective fermentation properties of a special dehydrated yeast.

Somogyi (10), Stark and Somogyi (11), and, more recently, Pan, Nicholson, and Kolachov (6) used pH to control the selective fermentation of maltose and glucose by baker's yeast.

Chemical methods have been used for the determination of sugars in fermenting doughs and breads, the most recent being the application of paper chromatography by Koch *et al.* (5).

[1] Manuscript received July 31, 1953. Presented at the annual meeting, A.A.C.C., May, 1954.

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Gas production methods are widely used in the cereal laboratory for the determination of the gassing power of flour and to study the rate of fermentation of yeast-leavened doughs. A manometric measurement of carbon dioxide produced in fermentation tests with yeasts would seem to provide a convenient and rapid means of determining the hexoses and maltose in yeast-leavened products. The present study was undertaken to develop the method and apply it to bread made by both the straight dough and sponge dough procedures.

Materials and Methods

Flours. Sufficient quantities of the flours of the following analysis were stored at room temperature in polyethylene bags.

	Hard Winter Wheat		*Spring Wheat*
	A	*B*	*C*
Moisture, %	13.7	12.7	13.4
Ash, %	0.46	0.47	0.38
Protein, %	11.5	11.2	12.0
Maltose No., mg./10 g.[1]	304	317	267
Gassing power,[1] mm., total 4 hrs.	438	437	412
Gassing power, mm., 4 to 5 hrs.	37	54	41
Type of bleach	Benzoyl peroxide, potassium bromate	Benzoyl peroxide, chlorine dioxide	Benzoyl peroxide, potassium bromate

[1] Determinations were made at 30°C. as described in Cereal Laboratory Methods (1).

Sugars. The sugars used were sucrose, D-glucose, and commercial dextrose hydrate containing 0.02%, 0.50%, and 8.9% moisture, respectively.

Yeasts. Two specially grown yeasts,[3] 2019 and 1428, which will ferment glucose, fructose, and sucrose but not maltose, were used.

Synthetic Nutrient Solution. The synthetic yeast nutrient solution described by Atkin, Schultz, and Frey (2), as modified by Koch *et al.* (5), was used.

Measurement of Gas Production. Gas evolution tests were conducted at 30°C. in pressuremeters equipped with mercury manometers as described in Cereal Laboratory Methods (1).

In tests on doughs, 10 g. of flour (14.0% moisture basis) were mixed with 0.3 g. of yeast and 7 ml. of distilled water. Where indicated, yeast nutrient salts and vitamins and source of available nitrogen were added.

The closed cups were placed in the thermostatic bath, the pressure

[3] Yeast 2019 was furnished through the courtesy of Fleischmann Laboratory and yeast 1428 by Annheuser-Busch.

was released at the end of 5 minutes, and readings were made at regular intervals. Unless otherwise noted the pressure was released at each reading.

Development of Manometric Procedure for Determination of Sugar

The selective fermentation property of the two yeasts was demonstrated by fermenting sucrose and D-glucose in a synthetic nutrient solution alone and in the presence of maltose at 30°C. Each pressuremeter contained 7.4 ml. nutrient solution, 300 mg. of the sugar, and 10 ml. of a 6% solution of the special yeast, making a total of 20 ml. As the following indicates, these yeasts do not ferment maltose but readily utilize sucrose and D-glucose and produce carbon dioxide from them in proportion to the fermentable solids available.

Carbon Dioxide as mm. Hg

Time, hours	*No Sugar*	*Sucrose, 300 mg.*	*D-Glucose, 300 mg.*	*Maltose, 300 mg.*
1	2	82	79	6
2	0	106	104	0
3	0	30	25	0
4	0	10	6	0
5	0	5	4	0
6	0	1	0	0
Total	2	234	220	6

These yeasts exhibit the same selective fermentation properties in doughs. As illustrated below, yeast 2019 readily ferments sucrose and hexose sugars present in flour A, or those produced by hydrolysis, but not the maltose formed by enzymatic action or maltose added to the dough. Results with baker's yeast are typical of a flour of average alpha-amylase activity.

Carbon Dioxide as mm. Hg

Hours	*Baker's Yeast*	*Yeast 2019*	*Yeast 2019; Maltose, 300 mg.*
¼	101	56	55
2	140	30	38
3	145	13	14
4	53	10	10
5	36	8	7
6	33	6	7
Total	508	123	129

Table I shows the application of special yeast 1428 to the evaluation of the fermentable solids contributed to a dough by various sug-

ars, as compared to inhibiting the amylase activity by decreasing the pH of the dough to 3.2, allowing it to stand 15 minutes, and increasing the pH to 5.6 with sodium carbonate (3) and then fermenting with baker's yeast.

TABLE I

FERMENTATION OF SUCROSE AND DEXTROSE HYDRATE IN DOUGHS BY 1428 YEAST, AND BY BAKER'S YEAST IN DOUGHS IN WHICH AMYLASE ACTIVITY HAS BEEN INHIBITED

Hours	Mercury Pressure, in mm.					
	No Sugar		Sucrose (300 mg.)		Dextrose Hydrate (300 mg.)	
	1428 Yeast	Baker's Yeast	1428 Yeast	Baker's Yeast	1428 Yeast	Baker's Yeast
1	50	81	117	119	115	120
2	26	29	127	144	118	138
3	16	10	57	61	38	44
4	10	7	22	16	18	7
5	10	5	12	9	11	4
6	7	5	10	5	11	1
Total	119	137	345	354	311	314

The amount of carbon dioxide produced from the dough containing sucrose is greater than from the dough containing dextrose hydrate, when fermentation is allowed to proceed to completion, by the amount, relatively, by which the fermentable solids present in the sucrose and formed by inversion exceed the fermentable solids in dextrose hydrate. The residual sugars in bread can be expected to reflect this same quantitative relationship.

Experiments were conducted to determine whether the sugars present in bread crumb could be fermented without extraction as is necessary in chemical methods for analysis of sugars in dough and bread. This was demonstrated by gelatinizing flour in the pressuremeter and fermenting the dough with and without added D-glucose, using yeast 2019. The dextrose was recovered in proportion to the amount added to the flour.

It was found more convenient to use a commercially available gelatinized wheat starch in further experiments. Ten grams were mixed with 10 ml. of 6% yeast solution and 10 ml. of water. As all tests were comparative, manometer readings were not corrected to atmospheric conditions. As shown in Table II, the added dextrose was recovered in proportion to the quantity present, the average being 74 mm. per 100 mg. dextrose. This cannot be assumed to represent the complete quantitative utilization of the sugars present in the fermenting solution to form carbon dioxide. The formation of glycogen, the production of organic acids – lactic, acetic, etc. – the utilization

TABLE II

FERMENTATION OF D-GLUCOSE BY 2019 YEAST IN PRESENCE OF GELATINIZED WHEAT STARCH

Dextrose Hydrate Added	Mercury Pressure, in mm.	
	Total	Per 100 mg. Dextrose
mg.		
0.0	6 [1]	
100.0	84	78
200.0	155	75
300.0	231	75
400.0	296	72
500.0	359	71
600.0	435	71

[1] A fermentation test using baker's yeast gave a value of 107 mm.

of carbohydrates by the yeast for cell growth, and the solubility of carbon dioxide in the fermenting system are factors which result in a substantial reduction of the carbon dioxide evolved as compared to that theoretically produced.

The amount of sugar originally present in a completely fermented solution or dough could be calculated from the mm. of pressure developed by using an average factor for the percentage recovered. However, as yeast varies and environmental conditions change, it is desirable to determine in each series of tests the amount of carbon dioxide evolved from a given amount of dextrose or sucrose when superimposed upon a completely exhausted solution or dough.

Selection of Bread Sample. Since the sugars in bread are in solution, their distribution will correspond with the moisture content of the crumb. Accordingly, in the present studies samples of crumb were taken as near the center of the loaf as possible and the moisture content determined with a 10-g. portion of a well-mixed sample of the crumb. This permits calculating the sugar content of the bread on any basis desired. In these tests results are reported on the dough basis, assuming the crumb moisture to be the same as in the bread tested.

Recovery of Sugar Added to Bread. To determine whether dextrose could be recovered quantitatively when added to bread crumb, Italian bread which was shown to contain no sugar fermentable by either 2019 or 1428 yeast was used.

A 13.5-g. portion of bread crumb, with and without various additions of D-glucose, was placed in the pressuremeter, and 7.5 ml. of water and 10 ml. of a 6% yeast suspension were added. The crumb was quickly and thoroughly mixed into a dough. Manometer readings

were made hourly until fermentation ceased. The results, in Table III, show that the Italian bread contained no sugars fermentable by 2019 yeast but large amounts of maltose were present, which were fermented by baker's yeast. The pressure developed was linearly related to the dextrose added, averaging 65 mm. Hg per 100 mg. D glucose.

TABLE III

SELECTIVE FERMENTATION OF DEXTROSE ADDED TO BREAD CRUMB WITH 2019 YEAST

Dextrose Hydrate Added	Mercury Pressure, in mm.		
	Total	Per 100 mg. Dextrose	Dextrose Hydrate Calculated[2]
mg.			*mg.*
0	0[1]	..	0
100	68	68	105
200	124	62	191
300	203	68	312
400	243	61	374
500	329	65	500
Average		65	

[1] With baker's yeast a value of 258 mm. was obtained.
[2] Based on an average of 65 mm. of Hg pressure developed per 100 mg. dextrose hydrate fermented.

Similar results were obtained on bread made in the laboratory without added sugar.

To demonstrate that the dextrose was completely exhausted by 2019 yeast, the pressuremeter cup was opened and the dough mass stirred vigorously. No further pressure was developed after the cup was replaced in the thermostat. In other experiments sucrose and dextrose were added to the fermented dough, after which carbon dioxide production started immediately at a rapid rate.

In another test, bread was baked from flour C by the sponge dough process with 6% maltose, the only sugar added at the dough stage. A sample of the crumb was fermented with 2019 yeast, in the manner described above, for 2 hours without any carbon dioxide being produced. At the end of that time 0.6 g. baker's yeast was added and fermentation started immediately.

These experiments show that the amount of hexoses present in bread may be determined conveniently by fermenting with 2019 yeast and then adding a known amount (preferably 100 mg.) of D-glucose and continuing fermentation. The quantity of hexoses present in the bread is then calculated by assuming a direct proportion between the mm. of pressure produced and the D-glucose present. This method makes it unnecessary to reduce manometer readings to standard conditions. Each experiment is complete and the carbon dioxide pro-

duced is in direct proportion to D-glucose added. Although it would appear possible to eliminate the addition of D-glucose after fermentation of the bread, by using an average value of mm. per 100 mg. dextrose, it is desirable to add the D-glucose in each test to have it fermented under the same conditions.

The amount of yeast was selected as 0.6 g. because some breads contain large quantities of sugars and an excessively long time would be required to ferment them completely if less were used.

Effect of Synthetic Nutrient Solution. While the use of synthetic yeast nutrient solutions has been demonstrated to be necessary in the fermenting of sugar solutions (Atkin, Schultz, and Frey, 2), dough and bread contain sufficient soluble nitrogen and thiamine for maximum utilization of the sugars present by the yeast. This was shown by using synthetic nutrient solution to replace the water added in the preparation of doughs made from bread containing various amounts of sugar and dextrose in the manner described above. There was a slight increase in carbon dioxide as indicated by pressuremeter readings, but, as all tests were affected by the same amount, it did not appear necessary to add the nutrient salts and it was not included in the procedure used in the following experiments.

Residual Sugars in Bread

Method. A 13.5-g. portion of crumb is selected from the center of a loaf of bread and mixed into a dough in the pressuremeter cup with 5 ml. of distilled water and 10 ml. of 6% suspension of a yeast that has been demonstrated not to ferment maltose. Fermentation is conducted at 30°C. and manometer readings are made every hour until fermentation has ceased. The pressuremeter is then opened and 100 mg. of D-glucose added. Fermentation is again allowed to proceed to completion, with manometer readings made every hour. The hexoses in the bread are then calculated by dividing the mm. of Hg corresponding to 100 mg. of D-glucose into the total number of mm. of pressure developed by fermentation of the sample.

Another 13.5-g. sample of the crumb is fermented in a similar manner with baker's yeast. The maltose content of the bread is obtained by substracting the mm. of Hg pressure produced by the special yeast from the total produced by the baker's yeast and dividing by the mm. of Hg corresponding to 100 mg. of maltose. The moisture content of the well-mixed sample of crumb is determined and results reported on any basis desired.

The bread should not be over 24 hours old unless it has been held at low temperatures. Both types of yeast must be as fresh as

possible to eliminate the possibility that bacterial decomposition may affect the results.

The method described was used in a series of experiments to ascertain whether the sugars present in bread can be recovered in proportion to the amount of fermentable solids introduced into the dough. Bread was made in the laboratory using the sponge process with 70% of the flour and 3% yeast in the sponge and 3½% milk solids nonfat, 3% shortening, 2% salt, and the indicated amounts of sucrose added to the dough. Results are given in Table IV.

TABLE IV

RECOVERY OF HEXOSES FROM BREAD MADE WITH VARIOUS AMOUNTS OF SUCROSE
(Results on Dough Basis)

Sucrose Added[1]	Hexoses in Dough[2]	Mercury Pressure	Hexoses in Bread[3]	Fermentation Loss
%	%	*mm.*	%	%
0.0	0.00	0	0.00	0.00
1.0	0.59	3	0.00	0.59
2.0	1.18	31	0.26	0.92
4.0	2.36	164	1.38	0.98
6.0	3.44	293	2.47	0.97
8.0	4.58	431	3.63	0.95

[1] On flour basis.
[2] Calculated from the quantity of sucrose added to the flour, with allowance for the increase in hexoses through inversion.
[3] Expressed as dextrose; fermentation of 100 mg. dextrose produced 88 mm. mercury pressure. Moisture content of bread crumb and dough were essentially equal.

No hexoses were present in the bread made with no sugar and with 1% sugar. These breads were small in volume and light in crust color. A small amount of hexose was present in the bread made with 2.0% sugar, and it was normal in volume but light in crust color. Higher amounts of sugar gave correspondingly higher percentages of residual sugars.

The amount of hexoses consumed by fermentation when an excess of sucrose is present was approximately 1% based on dough weight, which corresponds to 1.8% sugar based on the flour.

The maltose was determined in the manner described above in another series of breads made by the sponge process, with results shown in Table V.

Determination of Sugars in Bread Made with Sucrose and Dextrose Hydrate. Tests were also made comparing fermentable solids available in equal amounts of sucrose and dextrose hydrate, as this would be additional evidence of the application of the method to baking technology. The two sugars were compared in bread made in the laboratory by both the sponge and straight dough process at

several levels, with results shown in Tables VI and VII.

In all cases bread made with sucrose contained more hexoses than the bread made with the same percentage of dextrose hydrate. Fermentation loss was substantially the same with both sugars, approximately 2% of the sugar solids (flour basis) being consumed in fermentation. There was little difference in the maltose content of the breads made from the two sugars.

TABLE V

DETERMINATION OF MALTOSE IN SPONGE DOUGH BREAD BY SELECTIVE FERMENTATION
(Carbon Dioxide Expressed as mm. Hg)

Sucrose Added[1]	Mercury Pressure, in mm.			Maltose in Bread[2]
	Due to Yeast		Due to Maltose	
	2019	Baker's		
%				%
0.0	0	12	12	0.18
1.0	5	19	14	0.23
2.0	32	47	15	0.24
4.0	158	159	1	0.00
6.0	285	294	9	0.15
8.0	387	402	15	0.24

[1] On flour basis.
[2] Fermentation of 100 mg. maltose with baker's yeast produced 47 mm. mercury pressure. Moisture content of bread crumb and dough were essentially equal.

TABLE VI

HEXOSES AND MALTOSE IN SPONGE DOUGH BREAD MADE WITH VARYING AMOUNTS OF SUCROSE AND DEXTROSE HYDRATE
(Reported on Dough Basis)

Sugar Added[1]	Hexoses in Dough[2]	Mercury Pressure, in mm.		Hexoses Calculated[3]	Fermentation Loss	Maltose[4]	
		Baker's Yeast	2019 Yeast				
	%			%	%	*mm. Hg*	%
3% Sucrose	1.80	111	73	0.65	1.15	38	0.39
3% Dextrose Hydrate	1.56	72	46	0.40	1.16	26	0.27
6% Sucrose	3.52	292	258	2.29	1.23	34	0.37
6% Dextrose Hydrate	3.09	250	223	1.99	1.10	27	0.27
8% Sucrose	4.67	412	384	3.43	1.24	28	0.28
8% Dextrose Hydrate	4.09	340	308	2.76	1.33	32	0.32

[1] Flour basis.
[2] Hexoses present in the dough before fermentation; calculated from the quantities of sugars added to the flour.
[3] Fermentation of 100 mg. D-glucose with 2019 yeast produced 83 mm. mercury pressure.
[4] Fermentation of 100 mg. maltose with baker's yeast produced 72 mm. mercury pressure.

Table VII gives the hexose and maltose content of bread made in the laboratory by the straight dough process with 4% milk solids nonfat, 3% shortening, 2% salt, 0.5% yeast food (Arkady), 3% yeast, and various amounts of sucrose and dextrose hydrate.

TABLE VII

HEXOSES AND MALTOSE IN STRAIGHT DOUGH BREAD MADE WITH VARYING AMOUNTS OF SUCROSE AND DEXTROSE HYDRATE

(Reported on Dough Basis)

Sugar Added[1]	Hexoses in Dough[2]	Mercury Pressure, in mm.		Hexoses in Bread[3]	Fermentation Loss	Maltose[4]	
		Baker's Yeast	2019 Yeast				
	%			%	%	*mm. Hg*	%
3% Sucrose	1.79	106	8	0.0	1.79	98	1.06
3% Dextrose Hydrate	1.56	77	5	0.0	1.56	72	0.78
6% Sucrose	3.53	214	38	0.32	3.21	176	1.91
6% Dextrose Hydrate	3.06	153	14	0.12	2.94	139	1.51
8% Sucrose	4.67	340	161	1.36	3.31	179	1.92
8% Dextrose Hydrate	4.04	241	76	0.64	3.40	165	1.80

[1] Flour basis.
[2] Hexoses present in the dough before fermentation; calculated from the quantities of sugars added to the flour.
[3] Fermentation of 100 mg. D-glucose with 2019 yeast produced 88 mm. mercury pressure.
[4] Fermentation of 100 mg. maltose with baker's yeast produced 68 mm. mercury pressure.

No residual hexoses were present in breads made with 3% of the sugars. At higher levels the greater amount of fermentable solids in sucrose, as compared to dextrose hydrate, is evident in higher percentages of residual hexoses. More hexoses were consumed during fermentation than in the sponge process, and the maltose content of the bread was much higher.

Effect of Alpha-Amylase. The effect of alpha-amylase on residual sugars can be determined conveniently by the use of the method outlined. As shown in Table VIII, the addition of 1% of malted wheat flour (40 SKB units per g.) and of 0.1% of a fungal enzyme (5,000 SKB units per g.) did not increase significantly the hexose content of the bread but did increase the maltose content. The extent of this increase depends on the attackable starch present in the flour. This is indicated by the relatively small increase in maltose in the bread made with the fungal enzyme, which added over twelve times more SKB units than the malted wheat flour.

Determination of Lactose. The dough remaining after fermentation of sugars by baker's yeast after clarification can be used for gravi-

TABLE VIII

EFFECT OF AMYLASE SUPPLEMENTS ON REDUCING-SUGAR CONTENT OF SPONGE DOUGH BREAD MADE WITH 6% SUCROSE

Supplement[1]	SKB Units per g.	Mercury Pressure, in mm.		Hexoses[2]	Maltose[3]
		Baker's Yeast	2019 Yeast		
				%	%
None		273	259	2.28	0.16
1% Malted wheat flour	40	313	264	2.32	0.57
0.1% Fungal enzyme	5000	338	275	2.42	0.74

[1] Flour basis.
[2] Fermentation of 100 mg. D-glucose with 2019 yeast produced 84 mm. mercury pressure.
[3] Fermentation of 100 mg. maltose with baker's yeast produced 64 mm. mercury pressure.

metric determination of lactose from which the percentage of milk solids in the bread can be calculated. Table IX shows the percentage of lactose in bread made in the laboratory with the sponge dough process using 3½% milk solids nonfat.

TABLE IX

LACTOSE IN BREAD MADE BY SPONGE DOUGH PROCESS WITH 3½% MILK SOLIDS NONFAT AND VARIOUS LEVELS OF SUCROSE

Sucrose Present[1]	Lactose Percentage on Dough Basis	
	Calculated	Found
0.0	1.01	1.19
1.0	1.00	1.20
2.0	1.00	1.01
4.0	0.99	1.20
6.0	0.98	1.11
8.0	0.97	1.10

[1] Flour basis.

Determination of Residual Sugars in Commercial Bread of Known Sugar Content. The method was applied to the analysis of bread produced in two large commercial bakeries by the sponge dough process, using 3% and 7% sucrose and dextrose hydrate. In the bakery where the 3% sugar doughs were made, 75% of the flour was used in the sponge; milk solids nonfat, 4.0%; shortening, 3%; salt, 2.2%. In the 7% sugar doughs, made in another bakery, 60% of the flour was in the sponge, 3.0% milk solids nonfat, 4% shortening, and 2.25% salt.

The increase in sugar solids through the inversion of sucrose in the dough and the moisture content of commercial dextrose hydrate accounts for the difference in hexoses present in the dough before fermentation, as shown in Table X.

At the 3% level, a small amount of hexoses, fermentable by 2019

TABLE X

SUGAR CONTENT OF COMMERCIAL BREAD MADE BY THE SPONGE AND DOUGH PROCESS, WITH KNOWN AMOUNTS OF SUCROSE AND DEXTROSE HYDRATE

(Results Reported on Dough Basis)

Sugars Added[1]	Hexoses[2] in Dough	Mercury Pressure, in mm.		Hexoses[3]	Fermentation Loss	Maltose[4]
		Baker's Yeast	2019 Yeast			
	%			%	%	%
3% Sucrose	1.89	195	109	0.96	0.93	1.00
3% Dextrose hydrate	1.66	160	84	0.74	0.92	0.87
7% Sucrose	4.09	351	289	2.55	1.54	0.72
7% Dextrose hydrate	3.55	336	254	2.24	1.31	0.92

[1] Flour basis.
[2] Calculated hexose content of dough before fermentation.
[3] Fermentation of 100 mg. D-glucose with 2019 yeast produced 84 mm. mercury pressure.
[4] Fermentation of 100 mg. maltose with baker's yeast produced 64 mm. mercury pressure.

yeast, remained in the bread. This indicates that in this particular bakery 3% sugar or dextrose hydrate was sufficient to furnish the yeast with fermentable solids and leave a residue of hexoses in the bread; a larger quantity of hexoses remained in the bread made from sucrose than in that made from dextrose hydrate. Seven per cent sucrose and 7% dextrose hydrate left a higher residue of hexoses, the amount in the bread made with sucrose exceeding that in the bread from dextrose hydrate by 0.31%. This corresponds to 13.7% of the amount of hexoses found. The greater fermentation loss of the sugars in the doughs made with 7% sugar, compared to those made with 3% sugar, reflects differences in processing and shop conditions, such as percentage of sponge, dough time, and temperature.

The maltose content of the breads at both levels of sugars was much higher than in the laboratory sponge dough bread.

Sugar Content of Various Commercial Breads. The hexose content of 18 commercial breads of various types ranged from 0.0% to 3.54% and maltose from 0.15% to 2.85%, based on crumb moisture. Total sugar varied from 2.36% to 5.65%. The percentage of sugar used in the formula can be roughly estimated from the hexose content of the bread after making allowance for the sugar used during fermentation, keeping in mind that differences in formula and method of processing will cause considerable variation in this loss. The method is particularly useful in comparing different sources of fermentable carbohydrates and the effect of various enzyme systems on the quantity of residual sugars in bread.

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THE EFFECT OF SPICES ON YEAST FERMENTATION [1,2]

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ABSTRACT

Spices, at low levels, exhibited marked promoting effects on gas production in simple yeast-sugar suspensions as well as in more complex media including flour, yeast food, thiamine, and a synthetic mixture, containing asparagine, thiamine, pyridoxine, nicotinic acid, and inorganic salts. Yeast cell proliferation was not augmented by spices. In limited experiments with sweet doughs, spices promoted gas production in doughs made without shortening but not in those containing shortening.

At higher levels studied, several spices, particularly cinnamon, retarded gas evolution in simple yeast-sugar systems. The gas promoting effect of spices was counteracted by mustard flour which strongly inhibited yeast growth.

Little or no accelerating activity was found with the essential oils and some, such as cinnamon, retarded gas production.

The gas promoting factor was still present in spices after ether extraction, autoclaving, ethylene oxide treatment and prolonged boiling. The factor was not found in the ash or in charcoal-clarified filtrates from the boiled spices. More of the factor was found in the residues than in supernatant liquors following centrifugation of spice suspensions. An unexplained, progressive loss of the factor occurred during centrifugation particularly in boiled suspensions of caraway.

The gas promoting factor in ground spices is still unidentified except that it is relatively stable to heat and is not extracted by ether; nor does it appear in the ash.

Factors influencing gas evolution during yeast fermentation are of particular interest to cereal chemists. Gas promoting effects of various common bakery ingredients as well as many natural substances have been discussed and reviewed by Atkin, Schultz, and Frey (4).

Considering that spices are one of the most common and widely used classes of natural materials, there is surprisingly little quantitative information concerning their effects on yeast. Most of the published work has dealt with attempts to demonstrate preservative properties of spices and essential oils. Corran and Edgar (5) reporting on the preservative action of spices against yeast fermentation pointed out that certain spice materials actually stimulated the growth of yeast. Webb and Tanner (7) observed that yeasts could adapt themselves to low concentrations of spices and oils and in some instances appeared to be stimulated by them.

[1] Manuscript received May 28, 1953. Presented at Annual Meeting, May, 1953. Contribution from The R. T. French Co., Rochester, New York.

[2] This research was undertaken as a result of an inquiry from the American Institute of Baking to the American Spice Trade Association as to the effect of spices on yeast fermentation. It is part of a general program to develop basic scientific information on natural spices.

As far as the authors have been able to learn, however, no work has been published on the effect of spices on fermentation in bakery products. This is not surprising since the use of spices in the baking industry is primarily for flavor and their influence on fermentation is of secondary importance.

The present work was undertaken as a preliminary survey of the effect of spice materials on yeast fermentation as applied to the baking industry.

Materials and Methods

Eight spices, representing a cross section of botanical origins and morphological types, were selected. All samples were commercial grinds: caraway, nutmeg, and cardamom from whole seeds; ginger from the root; cinnamon from the bark; thyme from the leaf; mace from the dried fleshy aril or skin covering of the nutmeg; and mustard flour from the dehulled mustard seed. Three species of mustard, *Brassica alba, B. juncea, B. nigra,* and a commercial mustard flour blend were employed.

Essential oils of nutmeg and cinnamon and oleoresin of ginger were used in a study of the effect of spice fractions. Solubilized essential oils (containing added emulsifying agents) of caraway, nutmeg, cardamom, ginger, and cinnamon were likewise included. All were of commercial origin and were employed at levels indicated by the manufacturers to be equivalent to whole spices.

Boiled spice suspensions were prepared by the A.O.A.C. method for volatile oil in spices (2), except that distillation was continued for 17 hours. The volatile oil was removed in the process.

Ether-extracted spices were prepared by Soxhlet extraction for 22 hours.

Spice ash was obtained by the A.O.A.C. method (2) except that 10 g. samples were employed and the ashing time was increased to 5 hours.

Spices having reduced bacterial counts were prepared by treatment with ethylene oxide as follows: spices were placed in a chamber which was evacuated to 28 in. then ⅓ atmosphere of pure ethylene oxide admitted and left in contact with the spices for 16 hours. Ethylene oxide was then removed by evacuation.

Bacterial and mold counts on spices before and after treatment with ethylene oxide, were:

	Original Spice		*After Ethylene Oxide Treatment*	
	Bacteria/g.	*Mold/g.*	*Bacteria/g.*	*Mold/g.*
Cinnamon	52,750	10	25	0
Mace	23,000	10	20	0
Ginger	3,000,000	20	35	0
Caraway	22,000	10	10	0
Nutmeg	26,000	30	30	0
Cardamom	45,000	10	40	0

Gas production was measured by the A.A.C.C. volumetric method for doughs (1). Compressed yeast was used. Readings were recorded at 10 minute intervals but to save space only those values obtained at 180 minutes have been presented in the present paper. In all cases the data in the tables represent means of two or more determinations. The effect of agitation at 280 oscillations/minute and variation of pH, using phosphate-citrate buffers, was determined in representative simple systems and it was found that agitation had a similar effect on the rate of gas production in systems containing spices as in the control. The effect of spices was also determined to be relatively independent of pH over the range of 3.5 to 6.5. In unbuffered media, the decrease in pH during fermentation was approximately the same in the controls as in systems containing spices. In order to make as practical a comparison as possible of the effect of spices in systems of increasing complexity, some of which were not adaptable to continuous agitation, it was decided to employ static unbuffered systems throughout the extensive survey.

The basic fermentation medium used throughout most of the experimental work consisted of 2 g. sucrose, 1 g. yeast and 30 ml. distilled water. In a few studies, gas production was measured in 30 g. samples of sweet dough having the following basic formula:

	g.
Bread flour	300
Pastry flour	150
Sugar	87
Shortening (emulsifying)	100
Whole eggs	75
Liquid skim milk	190
Yeast	50
Salt	6.25
Malt (dry syrup, diastatic)	6.25

Yeast cell proliferation was measured, by microscopic cell counts using a haemacytometer with Spencer "bright line" counting chamber and double Neubauer ruling. A drop of well-mixed suspension was allowed to flow under the cover glass and yeast cells counted in the manner employed for red blood cells (6). Suspensions containing

approximately 1 g. yeast/liter were of appropriate concentration for convenient counting. Suspensions to be examined were treated with two drops of a 2% solution of mercuric chloride to stop yeast growth.

TABLE 1

EFFECT OF DIFFERENT LEVELS OF GROUND SPICES ON YEAST FERMENTATION IN SIMPLE SUGAR SOLUTIONS

(2 g. Sucrose, 1 g. Yeast, 30 ml. Water)

Spice	Quantity Added	Gas Evolved at 30°C. 180 Min.	Net Effect of Adjunct
	g.	*ml.*	*ml.*
Control		145	0
Caraway	0.1	246	+101
	0.25	279	+134
	0.5	334	+189
	0.75	347	+202
	1.0	358	+213
	5.0	405	+260
Cardamom	0.1	230	+ 85
	0.25	262	+117
	0.5	285	+140
Cinnamon	0.01	201	+ 56
	0.025	223	+ 78
	0.05	247	+102
	0.1	248	+103
	0.25	241	+ 96
	0.5	191	+ 46
	1.0	115	− 30
Ginger	0.1	232	+ 87
	0.25	277	+132
	0.5	283	+138
	0.75	317	+172
	1.0	281	+136
	2.0	217	+ 72
Mace	0.1	218	+ 73
	0.25	245	+100
	0.5	282	+137
Nutmeg	0.1	185	+ 40
	0.25	222	+ 77
	0.5	256	+111
Thyme	0.1	237	+ 92
	0.25	266	+121
	0.5	302	+157
	1.0	299	+154
Mustard flour[1]	0.25	25	−120
Mustard flour (*Brassica nigra*)	0.25	93	− 52
Mustard flour (*B. juncea*)	0.25	112	− 33
Mustard flour (*B. alba*)	0.25	23	−122
Mustard flour[1] plus caraway	0.1 0.25	118	− 27
Mustard flour[1] plus caraway	0.25 0.25	30	−115

[1] Commercial blend.

Results and Discussion

Effect of Ground Spices on Fermentation Rate. Gas production was accelerated by a variety of ground spices, with the exception of mustard, when added to simple mixtures consisting of 1 g. yeast and 2 g. sucrose in 30 ml. of water. Results are summarized in Table I.

Caraway was effective at all levels, bringing about an increasing rate of gas production with each increment of spice added from 0.1 g. up to 5.0 g. Cinnamon and ginger exerted similar accelerating effects at the lower spice levels, but both exhibited optimum spice concentrations, above which the promoting effect gradually declined. Cinnamon inhibited gas production at a level of 1.0 g.

Cardamom, mace, nutmeg and thyme promoted gas production over the limited range of concentrations investigated.

The enhanced gas production in spice media was not due to accelerated yeast cell proliferation. Yeast cell counts revealed 509,000 cells per cubic millimeter at the start of fermentation, with 549,000 and 533,000 for control and caraway media, respectively, after three hours' fermentation.

Mustard flours representing three different species of mustard as well as one commercial blend, all inhibited gas production. *Brassica alba* and the mustard flour blend which contained about 50% *B. alba*, limited gas evolution most effectively, while *B. nigra* and *B. juncea* were less inhibitory in the order given. The inhibition by mustard flour was so great as to counteract the marked gas promoting action of caraway as indicated at the bottom of Table I.

Effect of Ether Extraction. It was reasoned that the appreciable inhibitory effect demonstrated in the case of cinnamon was probably due to the essential oil present. To elucidate this point further, a series of experiments was carried out in which ether-extracted cinnamon and caraway were compared with their essential oils in a basic fermentation mixture consisting of 1 g. yeast, 2 g. sucrose, and 30 ml. water. The various spice fractions were used at levels calculated to represent certain concentrations of the original spice in each case. Also included were the essential oils of cardamom, ginger, and nutmeg as well as the oleoresin of ginger. The results of these fermentations are summarized in Table II.

Comparing ether-extracted caraway in Table II with the corresponding 0.25 g. and 0.5 g. levels, respectively, of original caraway in Table I, the gas promoting activity was lowered only slightly by ether extraction. The solubilized essential oil of caraway on the other hand evidenced no promoting activity at levels representative of 0.1 g. and

TABLE II

EFFECT OF DIFFERENT SPICE FRACTIONS ON YEAST FERMENTATION IN SIMPLE SUGAR SOLUTIONS

(2 g. Sucrose, 1 g. Yeast, 30 ml. Water)

Spice Fraction	Quantity Added	Gas Evolved at 30°C. 180 Min.	Net Effect of Adjunct
	g.	*ml.*	*ml.*
Control (no spice)		145	0
Caraway			
Ether-extracted[1]	0.19	267	+122
	0.38	311	+166
Essential oil (solubilized)[2]	0.005	139	− 6
	0.0125	142	− 3
	0.025	153	+ 8
Cardamom			
Essential oil (solubilized)[2]	0.002	135	− 10
	0.005	145	0
	0.010	146	+ 1
Cinnamon			
Ether-extracted[3]	0.097	256	+111
	0.24	264	+119
	0.48	270	+125
	0.97	258	+113
Essential oil (solubilized)[3]	0.002	133	− 12
	0.005	152	+ 7
	0.010	127	− 18
Essential oil[4]	0.002	145	0
	0.010	108	− 37
	0.020	65	− 80
Ginger			
Essential oil (solubilized)[2]	0.005	139	− 6
	0.0125	142	− 3
	0.025	145	0
Oleoresin[2]	0.005	148	+ 3
	0.0125	150	+ 5
	0.025	136	− 9
Nutmeg			
Essential oil (solubilized)[2]	0.006	145	0
	0.015	136	− 9
	0.03	120	− 25

[1] Equivalent to 0.25 g. and 0.5 g. of spice, respectively.
[2] Equivalent to 0.1 g., 0.25 g., and 0.5 g. of spice, respectively.
[3] Equivalent to 0.1 g. 0.25 g., 0.5 g., and 1.0 g. of spice, respectively.
[4] Equivalent to 0.1 g., 0.5 g., and 1.0 g. of spice, respectively.

0.25 g. of original spice. At the level equivalent to 0.5 g. of the original caraway, the solubilized caraway oil indicated slight, if any, acceleration yielding 153 ml. of gas as compared to 145 ml. for the control sample. The solubilized essential oils of cardamom and ginger behaved much like that of caraway.

The essential oil and solubilized essential oil of cinnamon as well as the oleoresin of ginger and solubilized essential oil of nutmeg all

exerted depressing effects as their concentrations were increased in the fermentation medium.

Ether-extracted cinnamon, unlike ether-extracted caraway, exhibited greater accelerating effects on gas production than the original spice. Evidently the gas promoting factor lies mainly in the ether-insoluble portion of the spice and is effective even at low spice levels. The inhibitory effect of the cinnamon oil in the unextracted spice is not manifested until higher spice levels are reached.

Effect of Boiling and Autoclaving. In further exploratory studies, spices were treated in a number of ways to learn more about the accelerating factor. Results are summarized in Table III.

TABLE III

EFFECT OF TREATED SPICES ON YEAST FERMENTATION IN SIMPLE SUGAR SOLUTIONS

(2 g. Sucrose, 1 g. Yeast, 30 ml. Water)

Treated Spice	Quantity Added	Gas Evolved at 30°C. 180 Min.	Net Effect of Adjunct
	g.	*ml.*	*ml.*
Control (no spice)		145	0
Caraway			
Boiled 17 hours[1]		245	+100
Autoclaved	0.25	248	+103
Ash	0.0161[1]	142	− 3
Cardamom			
Autoclaved	0.25	229	+ 84
Cinnamon			
Boiled 17 hours[1]		240	+ 95
Autoclaved	0.25	243	+ 98
Ethylene oxide-treated	0.5	200	+ 55
Ash	0.0080[1]	124	− 21
Ginger			
Autoclaved	0.25	257	+112
Ethylene oxide-treated	0.25	281	+136
Nutmeg			
Autoclaved	0.25	184	+ 39

[1] Samples equivalent to 0.25 g. spice.

Caraway and cinnamon were boiled 17 hours to distill off the volatile oils and then tested in the same yeast-sugar mixtures described previously.

The gas yield for caraway in a 3-hour fermentation dropped from 279 ml. in the original spice (see Table I, 0.25 g. level) to 245 ml. for the boiled caraway. This value was slightly less than the value of 267 ml. for ether-extracted caraway (see Table II) but was still quite significant when compared to 145 ml. for the control.

The corresponding values for cinnamon (Table I), ether-extracted cinnamon (Table II), and boiled cinnamon (Table III) were 241 ml., 268 ml., and 240 ml., respectively.

These results could be interpreted to mean that besides the effects due to removal of the volatile oils, there is in addition some slight measure of heat lability in the gas promoting factor.

This suggestion was borne out by data obtained with autoclaved (15 minutes at 15 lb.) caraway, cardamom, ginger, and nutmeg which yielded gas volumes of 248 ml., 229 ml., 257 ml., and 184 ml., respectively (Table III) as compared with 279 ml., 262 ml., 277 ml., and 222 ml., respectively for the corresponding original spices (Table I). It should be pointed out, however, that at the 0.25 g. level the effect of autoclaved cinnamon (Table III) like that of boiled cinnamon (Table III) was essentially unchanged as compared to the original spice (Table I).

Effect of Bacteria. The possibility that bacteria present in the spices might in some way contribute to the gas promoting effect was ruled out by the boiling and autoclaving experiments and it was also found that ethylene oxide-treated spices retained their full accelerating activity. Table III shows the gas yields obtained with ethylene oxide-treated cinnamon and ginger to be 200 ml. and 281 ml., respectively, as compared to 191 ml. and 277 ml., each for the original spices (Table I).

Effect of Spice Ash. The ash of caraway and cinnamon did not promote gas production. Yields of gas in both cases were less than the control value of 145 as shown in Table III.

TABLE IV

EFFECT OF RESIDUES AND SUPERNATANTS OF CENTRIFUGED SPICE SUSPENSIONS ON YEAST FERMENTATION IN SIMPLE SUGAR SOLUTIONS

(2 g. Sucrose, 1 g. Yeast, 30 ml. Water)

Spice Fraction[1]	Gas Evolved at 30°C. 180 Min.	Net Effect of Adjunct
	ml.	*ml.*
Control (no spice)	145	0
Cinnamon suspension	241	+ 96
Residue[2]	205	+ 60
Supernatant[2]	152	+ 7
Cinnamon suspension (boiled 17 hours)	240	+ 95
Residue[2]	192	+ 47
Supernatant[2]	168	+ 23
Caraway suspension	275	+130
Residue[2]	238	+ 93
Supernatant[2]	176	+ 31
Caraway suspension (boiled 17 hours)	245	+100
Residue[2]	170	+ 25
Supernatant[2]	168	+ 23

[1] All adjuncts equivalent to 0.25 g. spice.
[2] After centrifuging 20 minutes at 700 rcf.

Effect of Water Extracts. Inasmuch as all results indicated that the gas promoting factor resides mainly in the ether-insoluble or non-volatile portion of the spice, further studies were carried out on the effect of water extraction of spice. Table IV summarizes fermentation data obtained on residues and supernatant liquors following centrifugation of boiled and unboiled cinnamon and caraway suspensions, respectively.

The centrifuged residues in all instances possessed greater accelerating activity than the supernatant liquors. The centrifugates were not entirely clear and it seemed conceivable that with better separation even more of the promoting activity would have appeared in the residues. This assumption received some support when filtrates of boiled cinnamon, clarified with charcoal and diatomaceous earth were found to yield no more gas than the control medium. The possibility that a water-soluble, gas-promoting factor was adsorbed by the clarification process, however, should not be overlooked.

The residue of unboiled caraway suspension contained much more of the accelerating factor than did that of the boiled caraway. This is in line with the previous observation that some loss of the promoting factor occurs during heating.

However, the results in Table IV indicate that not all of the gas promoting factor had been satisfactorily accounted for in the centrifuged fractions, particularly in the boiled caraway residue.

Effect of Centrifuging Spice Suspensions. A possible explanation for this anomaly was found, when it was observed that boiled caraway suspensions showed marked loss in gas promoting activity during centrifuging. This mechanical effect is demonstrated graphically in Fig. 1, in which boiled caraway suspensions, centrifuged 0, 5, 20, and 60 minutes, respectively, and then resuspended are shown to have suffered a progressive loss in the gas promoting factor with time in the centrifuge.

This same effect was observed whether the centrifuged samples were resuspended with manual shaking, through the use of a Waring Blendor, or by trituration with a mortar and pestle. Saturating the suspension with nitrogen followed by centrifuging under nitrogen to preclude possible oxidative effects had no influence on the loss in activity during centrifuging. A curve for unboiled caraway is included in Fig. 1 to illustrate the loss of the gas promoting factor upon boiling prior to centrifuging.

Similar, but less marked, losses in gas promoting effect were observed when unboiled caraway and cinnamon suspensions were cen-

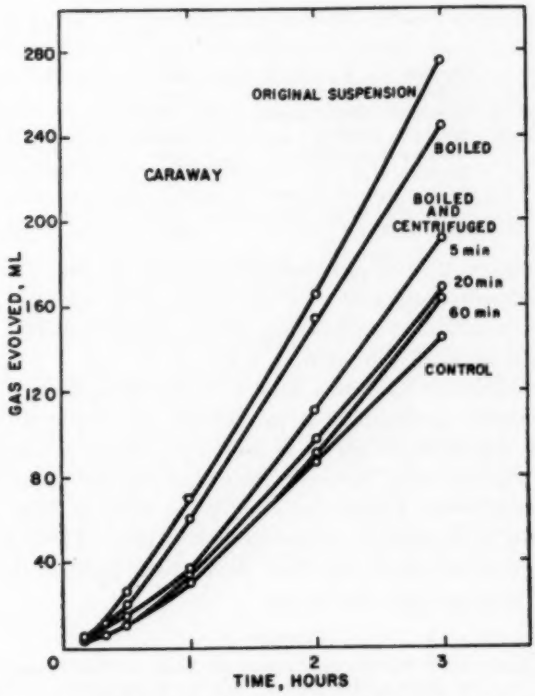

Fig. 1. Effect of centrifuging on the gas promoting factor in boiled caraway suspensions. Fermentations in mixtures containing 0.25 g. spice, 2 g. sucrose, 1 g. yeast, and 30 ml. water.

trifuged for 20 minutes. Values for unboiled caraway dropped from 275 ml. for a 3-hour fermentation down to 259 ml.; whereas, those for unboiled cinnamon decreased from 241 ml. to 185 ml.

No satisfactory explanation has been found for the loss in gas promoting activity which occurred as a result of centrifuging.

The occurrence of the gas promoting factor in the boiled and extracted spice residues suggests the possibility that the acceleration is due to some sort of surface phenomenon or even a mechanical supporting action.

Effect of Various Supporting Agents. The effect of various adjuncts which might conceivably behave in such a way was demonstrated in a simple basic fermentation mixture consisting of yeast, 1 g.; sugar, 2 g., and water, 30 ml. Compared to a control value of 138 ml. gas, the addition of 0.25 g. and 0.50 g. of various powdered substances yielded the following amounts of gas during a 3-hour fermentation.

	0.25 g.	*0.50 g.*
	ml.	*ml.*
Caraway	280	405
Charcoal (activated)	216	
Cellulose (filter paper)	173	
Asbestos (fibers)	170	
Diatomaceous earth	167	202
Kaolin	142	183
Glass (ground to pass No. 40 standard sieve)	142	
Corn starch	141	

The significantly greater gas promoting power of the caraway in the above experiments suggests that its accelerating activity is not due simply to a mechanical supporting action. Specific surface effects are not excluded as a possible explanation.

Effect of Spices in Complex Media. It has often been found that a substance which accelerates gas production in simple solutions may fail to show the expected effects in more complex fermentation media. Cinnamon and caraway, however, exhibited marked gas-promotion in the presence of flour. Cinnamon and ginger were notably effective in mixtures which included a commercial yeast food. Table V illustrates the influence of the above variables in the basic fermentation mixture containing yeast, sucrose and water.

TABLE V

FERMENTATION-PROMOTING EFFECTS OF SPICES IN THE PRESENCE OF SIMPLE MIXTURES OF BAKING INGREDIENTS

Adjuncts	Quantity Added	Gas Evolved at 30°C. 180 Min.	Net Effect of Adjunct
	g.	*ml.*	*ml.*
Control[1]		145	0
Caraway	0.25	279	+134
Cinnamon	0.25	241	+ 96
Ginger	0.25	277	+132
Flour	9.0	180	+ 35
Flour	9.0		
caraway	0.25	354	+209
Flour	9.0		
caraway	0.25		
salt	0.2	395	+250
Flour	9.0		
cinnamon	0.25	308	+163
Yeast food	0.0625	190	+ 45
	0.125	188	+ 43
	0.25	217	+ 72
Yeast food	0.125		
cinnamon	0.25	232	+ 87
Yeast food	0.125		
ginger	0.25	311	+166
Yeast food	0.25		
ginger	0.25	326	+181

[1] 2 g. sucrose, 1 g. yeast, and 30 ml. water.

The combinations of flour with caraway and cinnamon behave synergistically, promoting more gas than would be anticipated from the values obtained with the individual adjuncts.

Combinations of yeast food with cinnamon and ginger on the other hand promote slightly less gas evolution than expected from the yields accelerated by the separate adjuncts.

While data are not shown in Table V, combinations of 0.11 mg. thiamine and 0.25 g. caraway yielded 279 ml. gas, or no more gas than was promoted by caraway alone. The effect of caraway was pronounced even in the "complete" synthetic medium[3] described by Atkin, Schultz, and Frey (3). The total gas produced in three hours was: control (1.0 g. yeast, 2.0 g. sucrose, 30 ml. water), 154 ml.; "complete" medium, 236 ml.; and "complete" medium plus 0.25 g. caraway, 416 ml.

Only limited experiments have been carried out to determine the effect of spices on the gas production of doughs. Exploratory studies in Table VI showed that caraway had a notable accelerating effect in a sweet dough formula containing no shortening, but no appreciable effect on gas production in doughs containing shortening.

TABLE VI

INFLUENCE OF SPICES AND YEAST FOOD ON GAS PRODUCTION IN SWEET DOUGHS

Substrate	Gas Evolved at 30°C., 180 Min.
	ml.
Sweet dough:	
Control	150
Plus 0.25% caraway	144
Plus 0.67% caraway	146
Without shortening	141
Without shortening, plus 0.67% caraway	188

Initial baking experiments with cinnamon indicated desirable effects on crust color, volume, grain, and texture of sweet rolls.

Acknowledgment

The authors are grateful to Standard Brands, Incorporated, for supplying the yeast and the yeast food (Arkady) used in this work.

[3] Yeast, 1.0 g.; sucrose, 2.0 g.; water, 30 ml.; $NaH_2PO_4 \cdot H_2O$, 0.06 g.; $MgSO_4 \cdot 7H_2O$, 0.04 g.; KCl, 0.016 g.; asparagine, 0.2 g.; thiamine, 0.08 mg.; pyridoxine, 0.08 mg.; nicotinic acid, 0.8 mg.; and sodium citrate buffer (M/3) pH 5.5, 2.4 ml.

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A RAPID METHOD FOR THE DETERMINATION OF CEREAL LIPASE ACTIVITY [1,2]

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ABSTRACT

A rapid method for the determination of cereal lipase activity is described. The free fatty acids liberated in an emulsified reaction mixture buffered at pH 7.9 and maintained at 36°C. are determined by electrometric titration with standard alkali at two or three minute intervals. The lipase activity followed a zero order reaction rate for reaction times between ten and 60 minutes.

The deterioration that occurs in cereals or cereal products during storage is very often associated with an increase in fat acidity in the product (3, 11, 12). It has been suggested that this increase in acidity is the result of hydrolysis of lipid material by lipase enzymes, but this has never been tested because of the lack of a convenient method for the routine analysis of cereal products for lipase activity.

The lipase methods currently applied to cereal products are time consuming and difficult to run. Titrimetric methods have been described (6, 8) in which the enzyme is incubated overnight in the presence of an emulsion of a fat. The turbidity of the mixture makes it difficult to determine the end point of the titration. Wessel (10) described an experimental apparatus for continuous reading of hydrogen-ion concentration using a glass electrode rather than indicators which avoids the turbidity problem, but his method has never been applied to cereal products.

Rather than use an emulsified fat, a number of workers (7, 8) have used water soluble substrates such as triacetin, monobutyrin, or simple esters of short chained fatty acids. Such studies, however, are subject to the criticism that water-insoluble fats rather than these simpler, water-soluble esters are the natural substrates for lipases. The hydrolytic action of lipase on an organic compound such as triacetin or monobutyrin may or may not be related to its action on a fat. In the words of Sullivan, "The ideal substrate to use for a study of lipolytic enzymes of wheat would be wheat fat, provided that it could be uniformly emulsified by use of some surface active agent that had

[1] Manuscript received May 27, 1953. This paper reports research undertaken by the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned No. 430 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily the views or indorsement of the Department of Defense.

[2] Data in this paper presented at the annual meeting of the American Association of Cereal Chemists, May, 1953.

no inhibitory effect on the enzyme" (1). The method described in this paper approaches this ideal. This method is based upon the use of an emulsified fat as a substrate and the electrometric titration of the fatty acids liberated by enzyme action at appropriate intervals of time. The emulsified butterfat used in this work was very stable when sterilized and stored at 4°C. It was readily dispersible in water, and replicate emulsified samples give similar results. This substrate which had proved satisfactory for intravenous feeding (4) and for measuring the hydrolysis of fats by bacteria (5) seemed ideal for cereal lipase work.

Materials and Methods

Commercial samples of freshly milled wheat germ were used as a source of lipase in this study. The wheat germ was defatted by a 12 to 14 hours extraction with diethyl ether or benzene in a Soxhlet extractor. The defatted wheat germ was dried in the air, ground in a Wiley mill so that it would pass through a No. 20 sieve, and stored at 4°C. until used.

A butterfat emulsion served as the substrate for the wheat lipase. The emulsion was prepared according to the procedure of Goldman and Rayman (5). This involves the homogenization of a butterfat-water mixture in a Cherry-Burrell viscolizer to form an oil-in-water emulsion. Aztec 4135, a soy phospholipid obtained from the Associated Concentrates, Inc., was used as the stabilizer. The concentration of this emulsion stabilizer was 3%. After homogenization, the emulsion was sterilized, and stored at 4°C. until used. The particle size of the emulsion was very minute, being hardly discernible when viewed with an oil immersion lens of a microscope capable of 900× magnification. Several different batches of butterfat emulsion were used during the investigation with no difference observed in lipase activity due to the substrate.

The buffers used in the reaction mixture were either phosphate or borate (listed in Clark, 2). The buffers (0.5 *M*) were employed to prevent rapid and large changes in pH so that titration would not have to be performed too often, and thus make it possible to make more than one determination at the same time. Below pH 7.7, a phosphate buffer was used. Above, the borate buffer was used.

Prior to use, the wheat germ was allowed to attain room temperature. Lipase activity of the wheat germ was determined by placing 4.0 g. in a 250 ml. beaker, 100 ml. distilled water (heated to the bath temperature) added, and the mixture thoroughly stirred. Then 10 ml.

of buffer solution and 10 ml. of butterfat emulsion were added in that order. The mixture was stirred continually in a constant temperature bath at selected temperatures except when electrodes were inserted for pH determinations. While the reaction mixture was being stirred, the electrodes were placed in distilled water at bath temperature. In order to improve the sensitivity of the electrodes, they were periodically placed in 1 *N* hydrochloric acid for a few minutes.

A constant temperature bath equipped with a mercury thermoregulator was used in this research. The bath was capable of maintaining the temperature at ±0.01°C. A Beckman pH meter (line operated and connected to a voltage regulator, 115 v.) with outside electrodes was used for following the changes in acidity of the reaction mixture. Reliable results could not be obtained by using pH indicator solutions to determine the titration end point. It was impossible to obtain checks in titration values because of the difficulty in observing the color change of the indicator in the turbid reaction mixture which itself developed a greenish yellow color above pH 7. By the use of a 4 g. sample of the defatted wheat germ sufficient lipase activity was present so that changes in pH could be read on the pH meter for very short incubation periods.

During the reaction period, the pH of the mixture was kept as close as possible to the desired value by adding a 0.1 *N* sodium hydroxide solution as required to maintain the pH within approximately ±0.02 pH unit. The zero time of the reaction was taken 10 minutes after the addition of the emulsion, as this length of time was necessary for manipulation of the sample and equipment in the constant temperature bath and to bring the pH of the reaction mixture to the desired value by the addition of standard alkali.

Blank. The blank determination was made by first heating the wheat germ and water mixture in a boiling water bath for one hour with continuous stirring. After heating, the mixture was allowed to cool to the temperature of the constant temperature bath, the original volume restored with distilled water, and the buffer and emulsion added. The procedure described for the unheated reaction mixture was then followed.

Results and Discussion

Effect of Time on Lipase Activity. A time study was made of the lipolytic action of defatted wheat germ, and as can be seen from Fig. 1, the lipase activity followed a zero order reaction rate between 10 to 60 minutes reaction time.

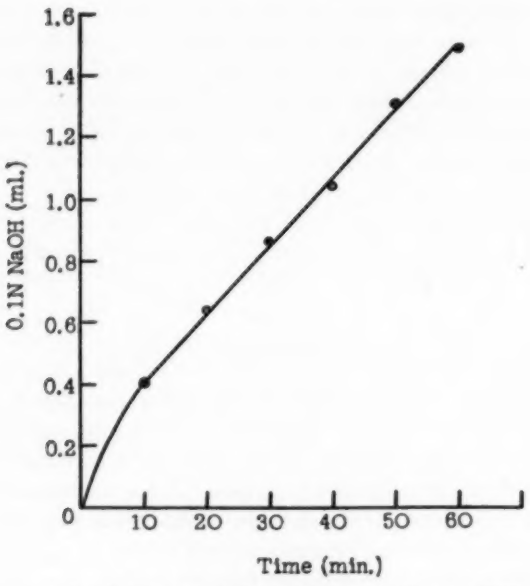

Fig. 1. Time courses of hydrolysis of butterfat by wheat germ lipase at 36°C. and pH 7.90.

TABLE I

TIME COURSE OF HYDROLYSIS OF BUTTERFAT BY WHEAT GERM LIPASE
(4 g. defatted wheat germ, 36°C., pH 7.9)

	0.097N NaOH					
Time-minutes	10	20	30	40	50	60
Exp. No.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
Total titre[1]						
1 (b)	0.53	0.90	1.18	1.45	1.72	1.97
2 (b)	0.68	0.98	1.26	1.50	1.88	1.95
3 (b)	0.62	1.08	1.30	1.52	1.89	2.10
4 (a)	0.48	0.90	1.29	1.54	1.82	2.00
5 (a)	0.62	0.85	1.18	1.48	1.81	2.08
6 (a)	0.57	0.96	1.35	1.49	1.80	2.10
7 (a)	0.58	0.98	1.28	1.51	1.80	2.03
Av.	0.58	0.95	1.26	1.50	1.82	2.03
Blank titre[1]						
1 (b)	0.14	0.28	0.38	0.46	0.51	0.54
2 (b)	0.25	0.38	0.47	...	0.52	0.55
3 (a)	0.15	0.30	0.37	0.45	...	0.50
4 (a)	0.16	0.32	0.38	0.46	...	0.55
5 (a)	0.18	0.30	0.39	0.46		0.55
Av.	0.18	0.32	0.40	0.46	0.52	0.54
Difference	0.40	0.63	0.86	1.04	1.30	1.49

[1] (a), Diethyl ether-extracted wheat germ; (b), benzene-extracted wheat germ.

Table I shows the reproducibility that can be expected from the method. There is good agreement for the titration values for the

longer periods of reaction time, while larger differences occurred for short reaction times as would be expected when using potentiometric techniques. Table I also shows that there is essentially no difference in the activity of the wheat germ whether it was defatted with diethyl ether or benzene.

The short total incubation time eliminated the necessity of using bacterial inhibitors because the bacteria are in the lag growth phase and remain inactive for at least the first two to three hours (9). Thus, little or no total activity can be attributed to the small number of bacteria that might be present at the start of incubation. However, it is important that the defatted wheat germ be handled in such a manner that any bacteria will not be in an actively growing stage at the start of the reaction.

Effect of Concentration on Lipase Activity. The results of an investigation on the effect of increasing the amount of wheat germ in the reaction mixture on the lipase activity, showed that essentially a straight line relationship existed for increase in activity with increase in concentration over the concentrations tested (Fig. 2).

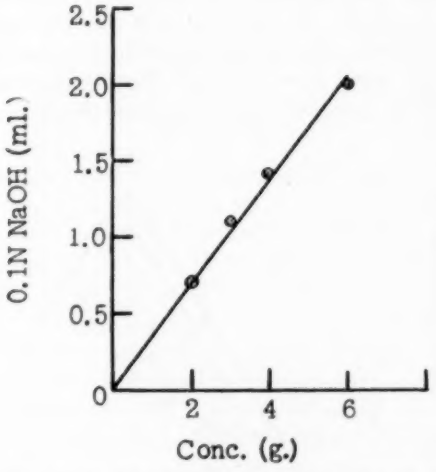

Fig. 2. The increase in the hydrolysis of butterfat with increase in wheat germ concentration. Reaction time, 1 hour, at 36°C. and pH 7.90.

Effect of Temperature on Lipase Activity. The determination of the variation in the lipase activity at different temperatures showed that the defatted wheat germ lipase had an optimum at 36°C. for a reaction period of one hour. As can be seen from Table II, the de-

TABLE II
EFFECT OF VARIATIONS IN TEMPERATURE ON LIPASE ACTIVITY
(At pH 7.9; Reaction time, 1 hour)

Temp. °C.	*0.097N* NaOH		
	Reaction Mixture	Blank Heated Control	Difference
	ml.	*ml.*	*ml.*
40	1.94	0.67	1.27
38	1.93	0.58	1.35
36	2.03	0.54	1.49
34	1.83	0.52	1.31
32	1.78	0.55	1.23
30	1.60	0.51	1.09
25.5	1.52	0.51	1.01

crease in activity on either side of the optimum temperature was quite gradual so that any slight variation in temperature will not have much effect on lipase activity. The temperature optimum is in fairly good agreement with the value of 38°C. obtained by Singer (7) and that of 35°C. obtained by Kretovich (6).

Effect of pH on Lipase Activity. The effect of different pH levels on lipase activity determined at 36°C. and 38°C. is shown in Fig. 3. The curves are quite similar; both temperatures showed rather sharp optimum values at pH 7.9 for a reaction time of one hour. In general, there was an increase in blank titration value with increase in pH level. At pH levels above 8.0 the blank values were quite high, and it was difficult to obtain good reproducibility at the higher pH levels (Table III).

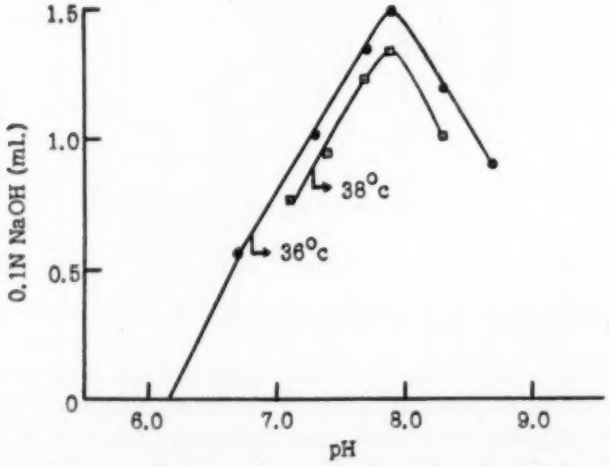

Fig. 3. Effect of variation in pH on lipase activity. Reaction time, 1 hour.

TABLE III

EFFECT OF VARIATIONS IN pH ON LIPASE ACTIVITY

(Reaction time, 1 hour)

pH	*0.097N* NaOH					
	36°C.			38°C.		
	Reaction Mixture	Blank Heated Control	Difference	Reaction Mixture	Blank Heated Control	Difference
	ml.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
6.2	0.00	0.00	0.00	...	...	...
6.7	0.67	0.06	0.61	...	...	...
7.1	...	...	...	1.20	0.43	0.77
7.3	1.32	0.30	1.02	...	...	...
7.4	...	...	...	1.42	0.47	0.95
7.7	1.93	0.58	1.35	1.79	0.56	1.23
7.9	2.03	0.54	1.49	1.92	0.58	1.34
8.1	2.40	1.03	1.37	...	...	...
8.3	2.47	1.27	1.20	2.04	1.03	1.01
8.7	2.60	1.70	0.90	...	...	...

Since a borate buffer was used at pH values above 7.7, and a phosphate buffer at lower pH ranges, the activity of the lipase was checked in both buffers at pH 7.7. At this pH, the lipase action in the phosphate buffer was 1.58 ml. of 0.1 *N* sodium hydroxide. In the borate buffer, the titration was 1.60 ml. This indicates little difference in lipase activity due to buffer effect.

Discussion

The method described in this paper is very simple and should be readily usable in laboratories working on cereal products. When working with wheat products, it is recommended that the lipase determination be carried out at pH 7.9 and at a temperature of 36°C. in order to obtain maximum activity. When working with other cereals, these conditions might serve as a starting point in the investigation. For most reproducible results it appears that reaction times of one hour or more should be employed.

The lipase activity of other fractions of wheat has been tested by this method, and it was found that the method was quite satisfactory for such determinations. It appears that at pH 7.9 wheat germ has over five times the activity of an equal quantity of patent flour. Preliminary results with emulsified wheat germ oil as the substrate for wheat germ lipase have indicated that the activity of the enzyme is quite similar to that obtained with the butterfat emulsion. Further investigations of this nature and on wheat germ lipase under conditions of greater purity are being conducted.

Acknowledgments

The authors gratefully acknowledge the assistance of Dr. M. M. Rayman and other members of the Stability Division of the Food Laboratories.

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EFFECT OF PROCESSING CONDITIONS ON THE EXPANDED VOLUME, COLOR, AND SOLUBLE STARCH OF PARBOILED RICE [1]

R. L. ROBERTS,[2] A. L. POTTER,[2] E. B. KESTER,[2] AND K. K. KENEASTER[3]

ABSTRACT

An increase in the severity of heat treatment during the parboiling process increased the degree of expansion of the dry milled rice (when heated in air), darkened the color, and increased the soluble starch content of the product. Steaming temperature exhibited the greatest influence on all of these factors, while steeping conditions and steaming time showed minor effects. Determinations of soluble starch and degree of expansion offer objective evaluation of the degree of parboiling, as affected by variations in processing.

The purpose of this investigation was to measure certain changes that occur in rice grains when they are parboiled, which may serve to indicate the severity of the parboiling treatment. Parboiling of rice is an ancient process used in the Orient, principally in India and Burma. In these areas the product is preferred to white rice by those accustomed to it (12). It has been estimated (9) that one-fifth of the world's annual rice crop is parboiled (about 170 million tons). Parboiling, as practiced in the Orient, has not been standardized to any extent. In some primitive forms of the process, rough (paddy) rice is simply soaked in warm water for several hours, then dried in the sun and milled. Originally, the intent was to facilitate removal of the hulls, and other incidental advantages were not recognized.

There have been several attempts to modernize and improve this process and in the past decade, as a result of considerable advancement in production details, three large commercial plants have been erected in the United States to produce parboiled rice. Several U.S. patents on parboiling, concerning various suitable equipment designs and processing details, have been issued. Typical examples are patents issued to Huzenlaub and Rogers, Jones and Brewer, and Yonan-Malek (4, 5, 13).

Parboiled rice is made commercially by steeping rough rice in warm water to increase the moisture content to about 30%, steaming the soaked rice, usually under pressure, to gelatinize the starch in the endosperm, drying the treated grain, and finally milling it to remove

[1] Manuscript received November 2, 1953. A report of work done under the Research and Marketing Act of 1946.

[2] Contribution from Western Regional Research Laboratory, Albany, Calif., Bureau of Agricultural and Industrial Chemistry, Agricultural Research Service, U. S. Department of Agriculture.

[3] Converted Rice, Inc., Houston, Texas.

hull and bran layers. Milled parboiled rice produced in this country has a light amber color and has a smooth glassy or translucent appearance, whereas ordinary milled rice is white and chalky. It is generally accepted that a large part of the water-soluble vitamins and minerals contained in the bran and embryo diffuse into the endosperm during the soaking and steaming steps of the parboiling process. In recent years the nutritional value of parboiled rice has been studied and reported by several investigators (1, 2, 7, 8, 9, 12). Milled parboiled rice has been shown to have two to five times the B-vitamin content of untreated white rice, depending on the processing conditions. Kik made a rather extensive study of the effects of various parboiling conditions on the vitamin content of the milled rice and on the milling quality (7, 8, 9). In addition to its increased nutritional value and resistance to insect infestation, parboiled rice has the advantage that the grains show little or no tendency to clump or become pasty when prepared for eating. For this reason many consumers prefer this type of rice. From the milling standpoint, the yields of whole-grain or head rice from parboiled paddy are substantially higher than from untreated rice. It is of interest that the additional costs of processing are largely offset by the increased yield of head rice, because whole-grain sells for about 50% more than broken rice.

The principal disadvantage of parboiled rice is the development of color during processing, which is not readily acceptable to those accustomed to highly milled white rice. Therefore, it would be commercially important to eliminate, or at least reduce, this color by some modification of the basic process or treatment to lighten the color. In general, past investigations on parboiled rice have been concerned with the effect of processing conditions on the vitamin and mineral content of the milled rice and to some extent with effect on milling quality. It has been shown (11) that dry parboiled rice will expand, in only a few seconds, to several times the original volume when heated in oil or air. Several factors influence the expansion of this form of rice. The increase in volume (ratio of volume of expanded product to original volume of rice) was reported (11) to vary with the temperature of the puffing medium and the moisture content of the milled parboiled rice. It was observed in later tests, however, that the maximum expanded volumes obtained under optimum conditions of temperature and moisture content of two lots of parboiled rice differed considerably. This apparent discrepancy could not be explained on the basis of varietal differences, shape of grains, or experimental error. It was proposed that the details of the parboiling process might also affect the degree of expansion.

Preliminary experiments showed that changes in the process, or the intensity of heat treatment, also affected the amount of soluble starch in the milled rice which is probably related to the degree of gelatinization. Intensity of heat treatment, as used herein, is construed to mean the total combined effects of the times and temperatures of both steeping and steaming steps and is referred to also as the degree of parboiling. It is clearly evident that there is a very large possible variation of parboiling conditions and that it is desirable to have some means of determining the effect of each phase of the process. The final effect of this treatment on the physical properties of the dry parboiled rice grain cannot be adequately evaluated simply by recording the processing data or by subjective appraisal. The fact that variations in parboiling conditions were found to affect the volume of the expanded product, obtained by heating parboiled rice in air at about 250°C., and the starch solubility, suggested the possibility that measurements of these factors might be used to indicate the degree of parboiling. In addition, color develops in the endosperm during the heat treatment, probably a result of the "browning reaction," and the degree of color varies with processing conditions. This report is a presentation of the influence of parboiling conditions on the expanded volume, color, and soluble starch.

Materials and Methods

Expanded Volume. The term "expanded volume" as used here is wholly arbitrary and is defined as the bulk volume of an expanded sample of rice divided by its initial volume. It was determined by heating portions of dry parboiled rice in an air blast at 250°C. (482°F.) for 16–18 seconds and measuring the increase in volume of the expanded product. All rice samples were held for 3–4 weeks at ordinary room temperature and humidity conditions in permeable containers to allow moisture contents to reach equilibrium (about 10.5%) prior to expanding. As the variation in moisture content of these samples was not more than 0.3% and the expanding temperature was held constant, it was considered that these factors would not be responsible for any measurable volume differences. Expanded volumes of replicates varied only 0.1 to 0.2 unit, which is well within the accuracy of measurement.

Color Difference. The color of the dry whole-grain parboiled rice was measured on the Hunter Color and Color-Difference Meter (3), an instrument designed for the rapid and precise evaluation of small color differences. It is a photoelectric tristimulus colorimeter meas-

uring color on three scales closely resembling the *L*, *a*, and *b* scales which are used to measure the perceptibility of differences between colors. The unit of color measurement for these three scales is the National Bureau of Standards unit of color difference devised by Judd (6). The magnitude of the unit was adjusted to make the color difference of one unit the maximum difference tolerable in the average commercial color match.

This meter measures color on three scales which may be considered coordinate axes of a three-dimensional system: Rd or reflectance which is converted to the related value *L* by reference to a conversion table or a calibration curve; *a* which measures redness when plus, zero when gray, greenness when minus; and *b* which measures yellowness when plus, zero when gray, blueness when minus. All of the *a* and *b* values for these rice samples were positive, and the color was in the area of yellow to slight orange. The color differences ΔE were calculated using color values for untreated white rice as the arbitrary standard. The values of ΔE are calculated as the vector sums of the differences in the three color scales, or

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}.$$

By this method of determining color differences it is possible to obtain objective measurements which obviate the disadvantages of visual appraisal, and which offer a more reliable comparison with other factors. In general, a ΔE value of one unit is about the limit of perceptibility.

Starch-Iodine Blue Value and Soluble Starch. Soluble starch was determined both by "blue value" and precipitation, followed by reaction with anthrone and sulfuric acid. The latter is more accurate, but is also more time-consuming and for practical purposes blue value is adequate.

The rice was ground in a Wiley mill to pass a 30-mesh screen. Two grams of ground rice were mixed with 200 ml. of hot water, 55°C. (131°F.). After standing at room temperature for 75 minutes with occasional stirring, the mixture was filtered using a Whatman No. 12 folded filter paper. The first 40–50 ml. of the filtrate were discarded. A 10-ml. aliquot of the filtrate was mixed with 1.0 ml. of an iodine solution (2 g. iodine and 20 g. potassium iodide in 1 liter of solution) and diluted to 100 ml. After 30 minutes the intensity of the starch-iodine blue color was measured in a Klett-Summerson colorimeter using a K66 filter and a 12.5-mm. tube standardized at 0 with 1.0 ml. of the iodine solution diluted to 100 ml. This reading is recorded as

the "blue value."

The starch in another 10-ml. aliquot of the filtrate was precipitated by adding 90 ml. of 95% ethyl alcohol and 25 mg. of potassium acetate and refrigerating at 3°C. (37°F.) for 16 hours. The precipitated starch was centrifuged from the supernatant liquid, dissolved in boiling water, cooled to room temperature, and diluted to 200 ml. The starch was determined in a 5-ml. aliquot of the diluted solution by the anthrone-sulfuric acid reaction (10).

In the initial phase of this study, a number of samples of parboiled long-grain rice (Rexoro variety) was prepared, covering a wide range of parboiling conditions. The time of treatment and the temperature of both the steeping and steaming steps were varied considerably. With few exceptions, the expanded volumes and color showed a close relationship to the severity of heat treatment. Although the starch data followed this same general trend, there was considerable dispersion, probably a result of the comparatively large variation in steeping conditions. Steaming temperature appeared to have the greatest effect on all these factors, particularly in the range of conditions normally encountered in commercial operations. However, steeping conditions and steaming time also showed minor effects.

To confirm earlier results, two duplicate series of parboiled long-grain rice samples, of the same variety, were prepared under more carefully controlled conditions. The temperature of steaming was the only variable in these series. All samples were steeped (as rough rice) for 165 minutes at 150°F. and steamed for 4 minutes at different temperatures. The several measurements were determined as indicated above.

Results and Discussion

A plot of color data against expanded volume (Fig. 1) shows an interesting comparison of the effects of parboiling on these two factors. It is observed that color development increases rapidly up to the point corresponding to a steaming temperature of about 212°F., with practically no increase in expanded volume of the treated grain over that of raw white rice. As the heat treatment increases from this point, the expanded volume goes up sharply with little increase in color. However, the color does increase to some extent when the retorting temperature is increased above 254°F. or when the soaking time or temperature is excessive.

It seems probable that most of the color developed in the endosperm during processing is a result of absorption of reducing sugars from the bran layer and embryo during steeping, followed by a re-

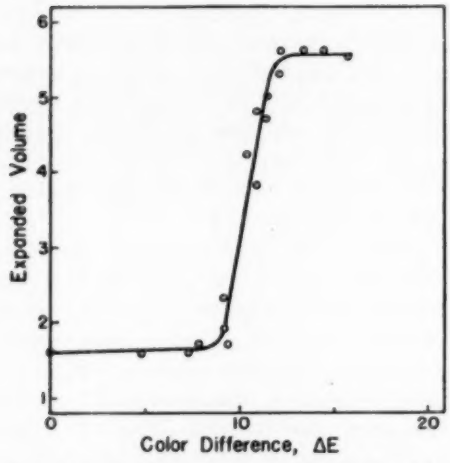

Fig. 1. Comparison of color of parboiled rice with expanded volume.

action with amino acids to produce browning. This absorption of reducing sugars probably increases with time and temperature of soaking. The subsequent browning effect is, of course, influenced by the severity of heat treatment in the steaming step. A more detailed study of the effect of parboiling conditions on the translocation of sugars is proposed for future consideration.

A plot of steaming temperature against expanded volume (Fig. 2) shows a good correlation. No appreciable increase in expanded volume

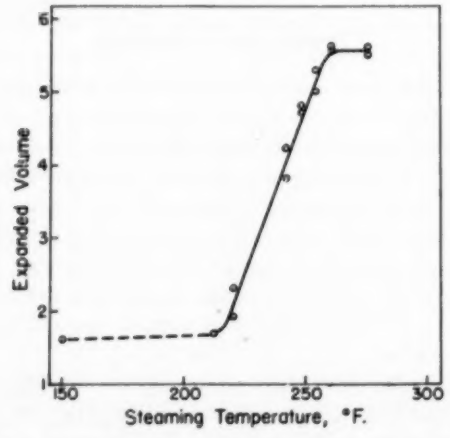

Fig. 2. Effect of steaming temperature on expanded volume of parboiled rice.

occurs until the temperature is raised to about 212°F. or higher. From this point, the volume increases rapidly with increased steaming temperature to a maximum of about 5.6 volumes and levels off at this value. The color also increases gradually with increased steaming temperature, which would be expected considering the general aspects of the browning reaction.

If in the process of parboiling rice the steeping and steaming temperatures are varied, the disorganization of the starch granules in the grain should vary, and it should be possible to observe these differences by a measure of the solubility of the starch within the rice. The results of starch analyses of the rice samples showed that this assumption was substantially correct. Results indicate that the starch-iodine value. The color also increases gradually with increased steaming temperature is increased and that the steeping temperature and time have a lesser effect on these values.

In Fig. 3 the soluble starch is plotted against the steaming tempera-

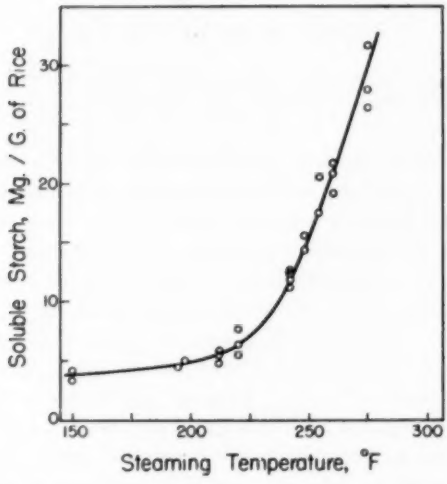

Fig. 3. Effect of steaming temperature on starch solubility of parboiled rice.

ture. Only the data obtained from the grains which were steeped at 150°F. for 165 minutes are shown. The increase of soluble starch with increasing steaming temperatures is small until about 212°F. Increasing the steaming temperature above this point produces a considerable increase in the soluble starch and there is no indication up to 275°F. of a maximum in the soluble starch as was found with the expanded volume. Blue value data showed this same general trend.

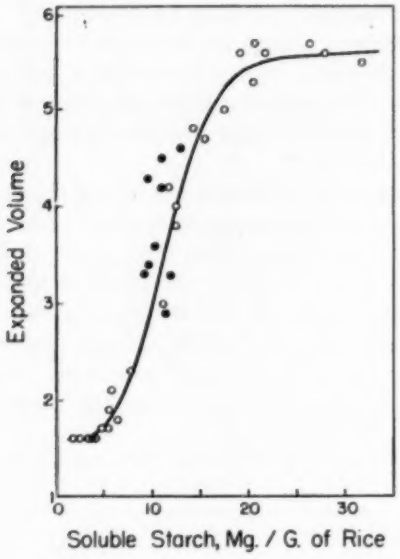

Fig. 4. Comparison of soluble starch with expanded volume of parboiled rice: ○, steeped at 150°F. for 165 minutes and steamed at various temperatures for 4 minutes; ●, steeped at various temperatures and times and steamed 4 minutes at 242°F.

Expanded volume plotted against soluble starch is shown in Fig. 4. Included in this figure are the data obtained from the samples steeped at 150°F. for 165 minutes and then steamed for 4 minutes at various temperatures. A measurement of the expanded volume up to a value of about 5, which corresponds to a steaming temperature of about 250°F., would more accurately indicate the parboiling conditions than a measure of the soluble starch. However, above this point a measure of the soluble starch is a better indication of the intensity of heat treatment. Also included in Fig. 4 are the results from samples steeped at different temperatures and times and steamed at 242°F. for 4 minutes. Higher steeping temperatures produced grains with larger expanded volumes but with only small differences in soluble starch. Therefore, in this region it would be preferable to measure the expanded volume to indicate differences in degree of parboiling as influenced by variation in steeping conditions.

Results of this work indicate that measurements of expanded volume or the starch solubility ("blue value", or total soluble starch) may be used as an objective evaluation of the degree of parboiling. Comparison of the intensity of heat treatment, or the separate steps of the parboiling process, with expanded volume, color, and soluble

starch may offer some explanation of the effects of various stages of processing on these characteristics.

Acknowledgment

We wish to extend our thanks to H. C. Lukens, of the Analytical, Physical Chemical and Physics Division of this Laboratory, for the color measurements.

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CHROMATOGRAPHIC ANALYSIS OF SUGARS IN BREAD[1]

THOMAS GRIFFITH AND JOHN A. JOHNSON[2]

ABSTRACT

The effect of concentration and sugar types in bread produced by a standard commercial sponge formula was determined by chromatographic methods. Four sugars, including sucrose, dextrose, levulose, and maltose, were used at concentrations of 2, 4, 6, 8, and 10% based on flour weight.

Maltose was readily fermented when added individually to doughs. However, when other sugar types were present, the residual concentration of maltose produced by amylolysis was constant and the fermentation of the maltose was negligible.

Qualitative analysis indicated that sucrose inversion was complete in the fermenting dough. The rates of fermentation of dextrose and levulose when both were present in the dough depended somewhat on the initial concentration of the sugars. At low concentrations the rate of dextrose fermentation was greater than the rate of levulose fermentation. When dextrose and levulose were added individually to doughs the rates of fermentation were essentially the same.

Until recently, relatively little information was available concerning the residual concentration of sugar in bread. Early analyses (14) were expressed as maltose, although several different types of sugar were known to be present. Morison (14) suggested that the inversion of sucrose in a fermenting dough was rapid. This was later confirmed by Geddes and Winkler (6) and by Koch, Smith, and Geddes (11). Rice (15), using copper reduction methods, showed that bread made with sucrose contained only a trace of sucrose. He also showed that the concentration of levulose was higher than that of dextrose, indicating that the latter was fermented at a faster rate than levulose. Dextrose and levulose were fermented more rapidly than maltose. These estimates confirmed the earlier work of Slator (16) and Hopkins and Roberts (7), who employed synthetic nutrient solutions.

The sugars that may be present in bread depend on those present in the flour, the breadmaking formula and process, and the extent of amylolysis. Under commercial methods of bread production, depending on the formula, dextrose, levulose, maltose, and lactose may be expected to be the predominant sugars. Traces of raffinose and another trisaccharide may be present (10). Bohn (3), using selective fermentation methods, found for commercial sponge doughs that the sugar utilized by yeast was approximately 2% of the flour weight depending on processing methods. Sugar added in excess of these

[1] Manuscript received December 3, 1953. Contribution No. 241, Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station, Manhattan, Kansas. Presented before the Tri-Section Meeting, American Association of Cereal Chemists, October 3, 1953, Manhattan, Kansas.

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amounts remained in the bread. Total residual sugar in commercial bread varied from 2.36 to 5.65% of the crumb weight (on crumb moisture basis).

An extensive study by Koch, Smith, and Geddes (11) using chromatographic technics to separate the various sugars revealed that dextrose was fermented at a greater rate than levulose when both sugars were present. Maltose fermentation was slight in straight doughs supplied with sufficient dextrose or sucrose, but in sponge doughs maltose was more readily fermented. Limited analyses of bread indicated that the amount and kind of residual sugar depended on initial concentration and type of sugar as well as processing method.

The importance of sugar type and concentration on the breadmaking process and quality of bread has been demonstrated by numerous workers (1, 2, 12, 13, 17). Barham and Johnson (1) demonstrated that equivalent concentrations of dextrose, levulose or sucrose, and maltose in the baking formula had slightly different effects on the quality of bread. At concentrations lower than 3% dextrose, the crust color was lighter than with equivalent concentrations of levulose or sucrose. This was assumed to be due to the more exhaustive fermentation of dextrose. At optimum concentration, bread made with dextrose tended to be slightly softer than bread made with levulose. The rates of fermentation and loaf volume were shown to be greatest with formulas which contained 3 to 6% dextrose. At sugar concentrations of 10% or more, gas production was partially inhibited, owing presumably to the plasmolysis of the yeast cell caused by the relatively high concentration of sugar in the water. Unfortunately, methods of analysis for sugars had not reached their present state of perfection and residual sugar analyses were not performed.

The present investigation has been made to study the effects of sugar type and concentration on the nature and amounts of residual sugars in commercial white bread using recently developed chromatographic methods of analysis.

Materials and Methods

Bread was baked, using a standard commercial sponge formula, by the procedure outlined by Johnson and Miller (9). The only variables were in the type and amount of sugar. Four sugars including sucrose, dextrose, levulose, and maltose were used at concentrations of 2, 4, 6, 8, and 10% based on flour weight. Following the baking, the bread was double-wrapped in waxed paper and kept frozen at −10°F. until analyses could be performed.

The sugars were extracted from 25 g. of bread crumb by refluxing

for 2½ hours with 80 ml. of 80% ethanol (10, 18). After the slurry was centrifuged, the alcohol was removed under reduced pressure and the concentrated solution made up to 25 ml. This solution was deionized by the batch method (19), using 0.5 g. each of Dowex 50[3] and Duolite A_4[4].

The sugars were separated by paper chromatography using a developing solvent consisting of a 7:1:2 mixture of propanol, ethanol, and water. The sugar solution, 5-12 μl., was spotted, on strips of Whatman No. 1 filter paper 4 in. x 21 in., with a micropipette. The concentration of the sugar ranged from 30 to 70 μg. The chromatograms were developed for 39 hours at constant temperature (82°F.).

Following the development of the chromatogram, the sugars on the strips were identified by use of one of the two spray reagents, aniline-diphenylamine (4) or 3,5-dinitrosalicylic acid (8). Quantitative determination of their concentration was carried out by the procedure of Dubois *et al.* (5).

Results and Discussion

Qualitative analyses of residual sugars in bread showed distinctly the presence of dextrose, levulose, lactose, and maltose. Compounds of lower R_f values than the disaccharides also were present. These compounds were not identified but could be small amounts of raffinose and another trisaccharide (11). No evidence for the presence of sucrose was obtained. The lactose in the bread was provided by the milk and, since it is not fermented by the yeast, the amount in all bread samples would be constant. Quantitative analyses for lactose were not performed.

The results of the quantitative analysis of the three major sugars, maltose, levulose, and dextrose, are shown in Fig. 1. The results are expressed as percent of residual sugar in the bread crumb on a 36% moisture basis.

Graph A shows the percent maltose added to the dough versus the percent maltose recovered in the crumb of the bread. There is a linear relationship between sugar added and the amount that remains in the crumb. It is evident that maltose is readily fermented when it is the only sugar available. A comparison of graph A with the maltose lines of graphs B, C, and D reveals that when other sugars are available the residual concentration of maltose, produced by amylolysis, is essentially constant and that the amount of fermen-

[3] Dow Chemical Company, Midland, Michigan.
[4] Chemical Process Company, 58 Sutten St., San Francisco, California.

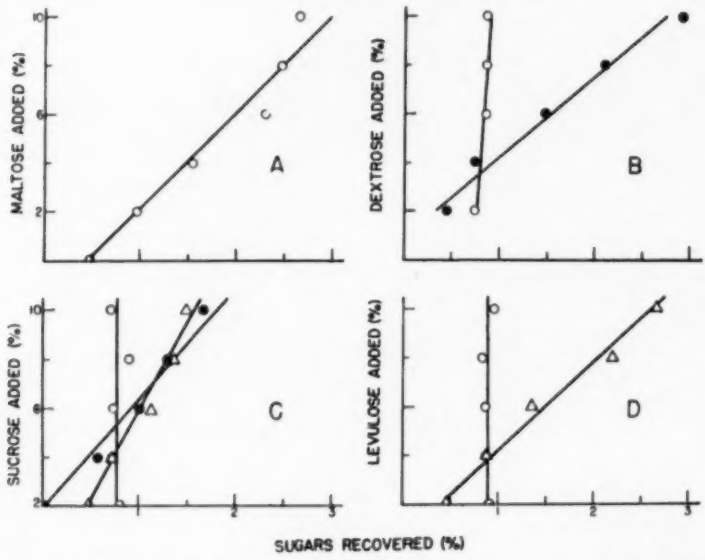

Fig. 1. Relationship between added sugar and residual sugar in baked bread. Legend: ○, maltose; ●, dextrose; ▲, fructose.

tation of this particular sugar is negligible. This confirms the data of other investigators (3, 11, 15, 17).

Graphs B and D represent the relationship between formula concentration and residual sugar in bread for dextrose and levulose, respectively. No evidence of residual dextrose in the bread made with levulose was observed. Likewise, the bread made with levulose had no residual dextrose. It is of interest that the residual amounts of dextrose and levulose in the bread crumb were essentially the same when added individually to the dough. This indicates that both sugars when present individually are fermented at essentially the same rate. In contrast, the data in graph C indicate that levulose is fermented at a lesser rate, depending on initial sugar concentration, than dextrose when both types are present. These results confirm the earlier work of Slator (16).

Acknowledgment

Acknowledgment is given to Corn Products Sales, Inc., for their financial assistance making this study possible.

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INTERRELATIONS AMONG BREAD DOUGH ABSORPTION, COOKIE DIAMETER, PROTEIN CONTENT, AND ALKALINE WATER RETENTION CAPACITY OF SOFT WINTER WHEAT FLOURS [1]

WILLIAM T. YAMAZAKI [2]

ABSTRACT

Studies on 448 samples of soft wheat flour milled from wheats grown at several locations for five consecutive crop years show that bread dough absorption is highly correlated with cookie spreading quality ($r = -0.873$) and with alkaline water retention capacity ($r = 0.874$), and that soft wheat flour protein content is associated to only a slight extent with cookie quality.

The similarity of factors apparently measured by absorption and alkaline water retention capacity is pointed out, and it is suggested that flour hydration is probably the basic factor associated with cookie quality.

Studies at the Soft Wheat Quality Laboratory, Wooster, Ohio, indicate that the bread dough absorption levels for varieties of soft winter wheat vary in a manner similar to that described by Finney (1) for hard winter wheats. However, absorption for the soft wheats is materially less than for the hard wheats, and is less highly correlated with protein content within a variety.

A similar relationship in the soft winter wheats exists for cookie quality, as measured by spread, and protein content. These facts seemed to warrant a more intensive study of these interrelations and of other physicochemical factors. The results of such a study, with particular emphasis on the relation between bread dough absorption and cookie diameter, are reported herein.

Materials and Methods

Flour samples used were Buhler-milled from 448 wheats representing 11 named varieties grown throughout the eastern United States in collaboration with plant breeders of the area for a period of five consecutive crop years, 1945 to 1949, inclusive, and covering a wide range in protein content and other soft wheat properties. Table I shows the number of samples and the ranges in protein content of the varieties used in this study.

Cookies were baked following the micro baking method described by Finney, Morris, and Yamazaki (2). The sum of diameters of the two cookies baked was used as the measure of quality, flours pro-

[1] Manuscript received September 23, 1953. Presented at the Annual Meeting, May, 1953. Cooperative investigation between the Section of Cereal Crops and Diseases, Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, and Department of Agronomy, Ohio Agricultural Experiment Station.

[2] Associate Chemist, Soft Wheat Quality Laboratory, Wooster, Ohio.

TABLE I

VARIETIES USED AND THEIR FLOUR PROTEIN CONTENT RANGES

Variety	No. of Samples	Flour Protein Range
		%
Kharkof	41	7.1 to 13.8
Purkof	40	6.8 to 12.9
Minturki	41	6.7 to 13.5
Kawvale	41	6.6 to 13.3
Clarkan	40	7.3 to 12.2
Trumbull	40	6.7 to 12.3
Fairfield	41	6.0 to 12.6
Thorne	41	6.3 to 12.0
Wabash	41	6.9 to 11.8
Blackhawk	41	7.4 to 14.0
Am. Banner	41	5.7 to 11.3
Total	448	5.7 to 14.0

ducing cookies with larger diameters being considered to have superior properties. Bread dough absorption requirements were determined according to Method III described by Finney (1), wherein initial absorptions were assigned to flours on the basis of variety and protein content and necessary adjustments made in subsequent bakes. Values for alkaline water retention capacity (AWRC) were obtained according to Yamazaki (3). All data are expressed on a 14% moisture basis.

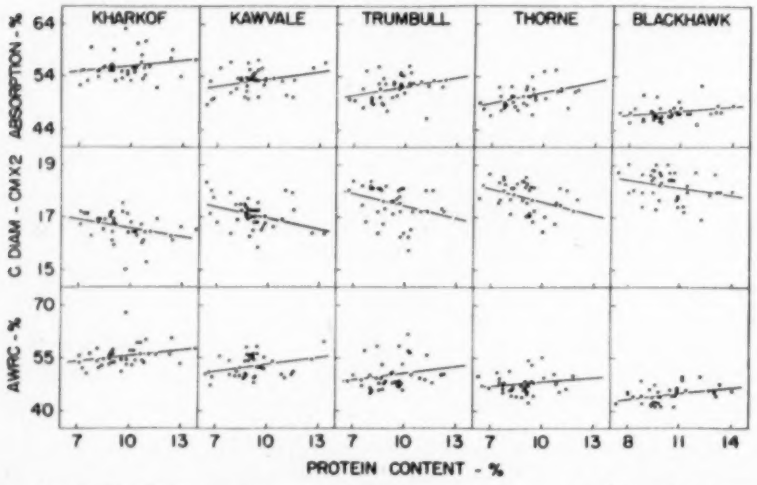

Fig. 1. Scattergrams and regression lines for protein content vs. bread dough absorption, cookie diameter, and alkaline water retention capacity, within several varieties of winter wheat.

Results

The relation between protein content on the one hand and bread dough absorption, AWRC, or cookie diameter on the other for five representative varieties grown over five crop years is presented in Fig. 1. The corresponding correlation coefficients are given in Table II.

TABLE II

CORRELATION COEFFICIENTS FOR PROTEIN CONTENT VS. BREAD DOUGH ABSORPTION, COOKIE DIAMETER, AND ALKALINE WATER RETENTION CAPACITY (AWRC) WITHIN SEVERAL VARIETIES OF WINTER WHEAT

Variety	n	Protein Content versus:		
		Absorption	Cookie Diameter	AWRC
		r	r	r
Kharkof	41	0.22	—0.30*	0.26
Kawvale	41	0.32*	—0.35*	0.30*
Trumbull	40	0.31*	—0.32*	0.20
Thorne	41	0.48**	—0.39*	0.16
Blackhawk	41	0.27	—0.30	0.37*

* Significant at 5% level.
** Significant at 1% level.

Although there appear to be trends toward increasing absorption and AWRC values and decreasing cookie diameter with increasing protein content, the regression lines are almost horizontal (Fig. 1) and the correlation coefficients are not significant or are significant at the 5% level only, with one exception.

It thus appears that variety, season, location, and other factors are exerting materially greater influences than protein content on cookie diameter, bread dough absorption, and AWRC. This is in contrast to the relation between protein content and such determinations as acid and alkaline viscosity, and mixogram area. For example, it has been found at the Soft Wheat Quality Laboratory that the correlation coefficient for flour protein content and acid viscosity for 56 samples of Thorne, grown over eight crop years and representing a wide range in protein content (6.3 to 11.8%), is 0.93. The r value for protein content and mixogram area for the same samples is 0.91[3].

The similarity of varietal scattergrams for protein content vs. AWRC and absorption suggested the existence of a relationship between absorption and AWRC. Further, since AWRC is highly correlated with cookie diameter (3), absorption could also be related to the latter.

[3] Unpublished data.

Accordingly, the interrelations for bread dough absorption, cookie diameter, and AWRC were determined for the same five varieties. Results are shown graphically in Fig. 2 and correlation coefficients are presented in Table III.

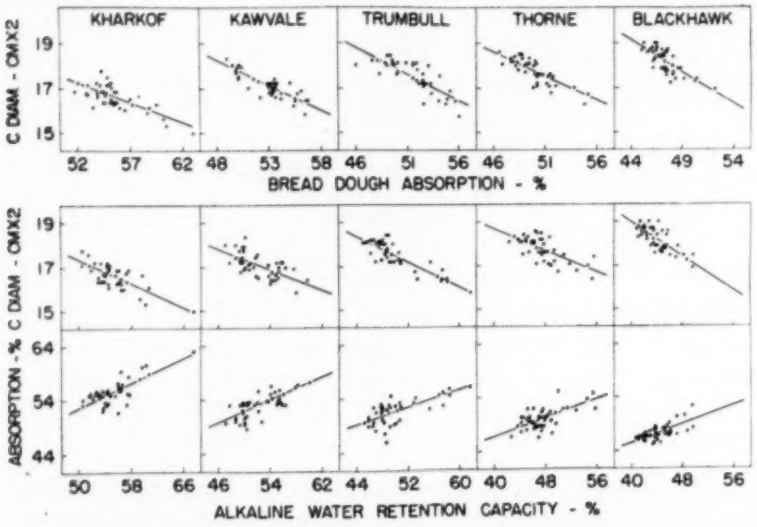

Fig. 2. Scattergrams and regression lines for bread dough absorption vs. cookie diameter and for alkaline water retention capacity vs. absorption and cookie diameter, within several varieties of winter wheat.

TABLE III

CORRELATION COEFFICIENTS FOR ABSORPTION VS. COOKIE DIAMETER AND ALKALINE WATER RETENTION CAPACITY (AWRC), AND FOR AWRC VS. COOKIE DIAMETER, WITHIN SEVERAL VARIETIES OF WINTER WHEAT

Variety	n	Absorption versus:		AWRC vs. Cookie Diameter
		Cookie Diameter	AWRC	
		r	r	r
Kharkof	41	−0.76***	0.75***	−0.73***
Kawvale	41	−0.84***	0.74***	−0.63***
Trumbull	40	−0.80***	0.67***	−0.85***
Thorne	41	−0.73***	0.63***	−0.64***
Blackhawk	41	−0.73***	0.69***	−0.77***

*** Significant at 0.1% level.

The very highly significant correlation coefficients are indicative of a high degree of association among all of the three factors.

Since the coefficients were substantially alike for all varieties the

absorption results were first pooled for each year regardless of variety, protein content, or source of sample and correlated with cookie diameter and AWRC, and finally regardless of crop years to give over-all correlation values. Very highly significant correlation coefficients were obtained in each case (Table IV).

TABLE IV

CORRELATION COEFFICIENTS FOR ABSORPTION VS. COOKIE DIAMETER AND ALKALINE WATER RETENTION CAPACITY (AWRC), AND FOR AWRC VS. COOKIE DIAMETER, WITHIN EACH OF SEVERAL CROP YEARS

Crop Year	n	Absorption versus:		AWRC vs. Cookie Diameter
		Cookie Diameter	AWRC	
		r	r	r
1945	88	−0.886***	0.869***	−0.863***
1946	98	−0.879***	0.884***	−0.892***
1947	66	−0.874***	0.917***	−0.881***
1948	97	−0.916***	0.899***	−0.864***
1949	99	−0.916***	0.927***	−0.936***
1945–1949	448	−0.873***	0.874***	−0.857***

*** Significant at 0.1% level.

TABLE V

FIVE-YEAR MEANS FOR ABSORPTION, AWRC, AND COOKIE DIAMETER, WITHIN EACH OF 11 VARIETIES OF WINTER WHEAT, AND CORRELATION COEFFICIENTS USING VARIETAL MEANS

Variety	Absorption	AWRC	Cookie Diameter
	%	%	$cm. \times 2$
Kharkof	55.54	55.20	16.59
Purkof	56.57	59.78	16.30
Minturki	51.31	48.92	17.56
Kawvale	52.99	52.32	17.08
Clarkan	51.66	52.48	17.50
Trumbull	51.57	50.05	17.48
Fairfield	50.58	49.36	17.87
Thorne	50.07	47.63	17.74
Wabash	49.14	46.87	18.15
Blackhawk	47.33	44.27	18.16
Am. Banner	47.06	44.61	18.07

Relationship	n	r
Absorption vs. cookie diameter	11	−0.97***
AWRC vs. cookie diameter	11	−0.95***
Absorption vs. AWRC	11	0.97***

*** Significant at 0.1% level.

To determine how varietal rankings were influenced by these relationships, five-year varietal means were calculated for all tests and correlated. Table V presents the mean values and correlation coefficients obtained using these means.

The high r values indicate that the varieties are being ranked quite well for all relationships but absorption appears to be slightly more accurate than AWRC in placing varieties according to their cookie baking potentialities for these samples.

Discussion

The data obtained in this study for protein content vs. bread dough absorption for soft wheat varieties show a general lack of significant correlation and almost horizontal regression lines. Finney (1) has found that for hard winter wheats, protein content is a good index of absorption within a variety, but for flours of less than 10% protein content[4] he has found that the relationship no longer holds, and a constant absorption may be used for a variety as a first approximation. Thus, for both soft wheats and low-protein hard winter wheats, bread dough absorption is not a function of gluten content or quality alone, but also of other hydrophilic agents.

Bread dough absorption data for the soft wheats tested indicate that the quantity of water required to form a dough of proper consistency is essentially a varietal property modified by factors other than protein content (Table III; top row, Fig. 1). Cookie diameter also appears to be a varietal characteristic, similarly affected by other factors. For example, although most of the diameters for Kharkof are between 16.3 and 16.8 cm., an occasional one may be as large as 17.5 cm.; and for Thorne most diameters are in the range of 17.5 to 18.2 cm., but one may be as small as 16.4 cm. Much of this variability appears to be due to seasonal effects, since most of the samples for any one crop year had diameters falling within fairly narrow limits.

Although it would not be practical to determine the extents of the relationships among bread dough absorption, AWRC, and cookie diameter for samples of an individual variety for any given crop year, such a procedure is possible where samples of a variety for several crop years are pooled or where all varieties are included for any crop year, because environmental and quality variations give wider ranges in test values. Very highly significant correlation coefficients are obtained in all cases tested, demonstrating the similarity of factors being measured. Thus, bread dough absorption and AWRC appear to be measur-

[4] Private communication from K. F. Finney.

ing cookie quality directly in spite of the presence of such variables as protein content, season, location, or variety (last row, Table IV). The two physicochemical procedures give good indications of varietal cookie baking potentialities, provided a sufficient number of samples is tested (Table V). When samples of individual varieties are tested, the correlation coefficients are not so high, owing to decrease in range without a corresponding decrease in sample variability.

The high r value obtained for bread dough absorption vs. AWRC permits the use of the latter test to estimate fairly accurately the absorption requirement of a soft wheat flour.

In obtaining the correct bread dough absorption, the quantity of water used is adjusted until dough consistency is optimum. There are cases when soft wheat flour doughs differ in elasticity owing to protein content and variety, but, on the whole, it is believed that doughs have similar plasticities at optimum absorption. The water may be considered as being held to flour by sorptive and cohesive forces in a series of layers. With increasing distance of the water molecules from the centers of attraction, the bonds become weaker, the outermost layer being held less strongly than any other layer. This outermost layer of water is capable of contributing more than any other layer to dough consistency or plasticity through its lubricating action. Since dough plasticity at optimum absorption appears to be similar for different flours, it would appear that the attractive force between water molecules at the outermost layer and flour are similar for all doughs.

An essential feature of the AWRC test is the centrifugation of a flour suspension in sodium bicarbonate solution. The result of this action is the packing of the solid material to which water is sorbed with varying degrees of tenacity. The total quantity of water adhering to flour particles is determined by the amount enclosed by the outermost layer. Since the same relative centrifugal force (r.c.f.) is applied to all samples, the attractive force between the outermost layer of water and flour particles is counteracted to the same extent in all cases. Although different flours may retain varying quantities of water, the forces involving the outermost layer of water are similar.

In the AWRC and bread dough absorption procedures, determinations are being made of the quantity of water held by flour under conditions of the applief r.c.f. in the former and a uniform handling consistency in the latter. Thus the two methods are measuring similar hydration characteristics in the flour-water systems, and since results of both correlate highly with cookie diameter, cookie quality would also appear to be a function of the delimited water sorption, or re-

tention, capacities of soft winter wheat flours.

Acknowledgment

The author is indebted to Mr. K. F. Finney, Hard Winter Wheat Quality Laboratory, Manhattan, Kansas, for a critical review of the report and to Mr. C. E. Bode, Soft Wheat Quality Laboratory, Wooster, Ohio, for the use of certain data.

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GRAIN STORAGE STUDIES. XIII. COMPARATIVE CHANGES IN RESPIRATION, VIABILITY, AND CHEMICAL COMPOSITION OF MOLD-FREE AND MOLD-CONTAMINATED WHEAT UPON STORAGE [1]

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ABSTRACT

Experiments with a western white wheat, free from internal molds, have made it possible to secure direct experimental evidence on the relative magnitudes of deteriorative processes inherent in the seeds themselves and those produced by the normal microflora on and within the seed.

The respiratory rates of mold-free wheat at 35°C. and moisture levels ranging from 15 to 31% were low and constant with time. In contrast, the respiration of the moldy wheat markedly increased after a few days. Loss in viability was enhanced by mold growth, although all samples stored at moisture levels exceeding 18% were nonviable. Large increases in fat acidity and decreases in nonreducing sugars occurred in the moldy samples but only slight changes were observed in the mold-free wheats.

These studies provide direct evidence of the importance of molds in the deterioration of stored grain.

Previous studies in these laboratories have produced evidence that molds are primarily responsible for the respiration, heating, and chemical deterioration of grain stored at moisture contents normally encountered in commercial practice (2, 3, 5, 7, 8, 9). The so-called "critical moisture values" where respiration increases rapidly and biochemical changes become more pronounced correspond to the minimum relative humidity (approximately 75%) at which molds, normally present on and in the seeds, will grow. Moreover, suppression of mold growth with fungistatic agents prevents the sharp increase in respiration rate, decrease in viability, and marked chemical deterioration which invariably occur at favorable temperatures when grain is stored at moisture levels exceeding the critical range (6). On the basis of these results it was inferred that at any moisture level below that required for seed germination, the respiration of the dormant seed is related to moisture content but remains relatively constant at any given moisture content.

In past studies on the over-all respiratory activity of stored grain, the production of carbon dioxide by contaminating microorganisms

[1] Manuscript received December 18, 1953. Presented at the Annual Meeting, May 1952. Paper No. 3052 of the Scientific Journal Series, Minnesota Agricultural Experiment Station. Cooperative investigations between the Departments of Agricultural Biochemistry and Plant Pathology and Botany, University of Minnesota, St. Paul, Minnesota.

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was never distinguished experimentally from that of the dormant grain. Sterile, viable seed would be necessary to secure direct evidence concerning the relative significance of seed and mold respiration at different moisture contents. Recently, a sample of western white wheat of high viability was located which was entirely free of internal mold infection; accordingly, it could be rendered mold-free by mild treatment with sodium hypochlorite without impairment of viability. Using wheat treated in this manner and wheat from the same lot inoculated with common storage molds, it should be possible to evaluate directly the contribution made by the grain to the total respiration. This paper deals with determinations of respiratory rate, viability, mold count, fat acidity, and sugar content of mold-free and mold-contaminated wheat stored at several moisture levels.

Materials and Methods

A western white wheat (Elgin) of 95% viability, grown under irrigation in Oregon, was cleaned, immersed in 1% sodium hypochlorite solution for 1 minute, drained free of liquid, air-dried in a vacuum desiccator, and analyzed for moisture. Subsamples were conditioned to a series of seven moisture levels (14.9–30.7%) with appropriate volumes of 1% sodium hypochlorite solution or sterile water. A corresponding set of subsamples was conditioned to the same moisture contents with water containing mold spores commonly found on wheat. Moisture values were determined by the two-stage air-oven method prescribed by the U. S. Department of Agriculture (11).

Respiratory rates were determined at 35°C. employing 100 g. of each sample under controlled aeration in the apparatus described by Milner and Geddes (7). Cotton plugs were employed in the air lines leading to and from the mold-free samples to prevent contamination. The respired gases were measured and analyzed for oxygen and carbon dioxide at appropriate intervals with the Haldane-Henderson apparatus (10). At the end of the incubation period (19 days) the samples were analyzed for moisture. The mold count was determined according to the method described by Christensen (4). Viability of the wheat was ascertained by spreading the kernels on salt-free malt agar in Petri dishes and counting those which had germinated after 4, 5, and 6 days at room temperature. The samples were also tested for viability by the Minnesota State Seed Testing Laboratory. Fat-acidity, reducing and nonreducing sugars were determined by the methods outlined for wheat flour in Cereal Laboratory Methods (1).

Results

Respiratory Rate. The rates of carbon dioxide production by the

various samples during incubation at 35°C. are shown in Figs. 1 and 2. The respiration of the mold-free samples was comparatively low and essentially constant with time, even at the highest moisture level employed (30.8%). In striking contrast, the mold-infected samples stored at moisture contents of 16.0% and higher gave the typical

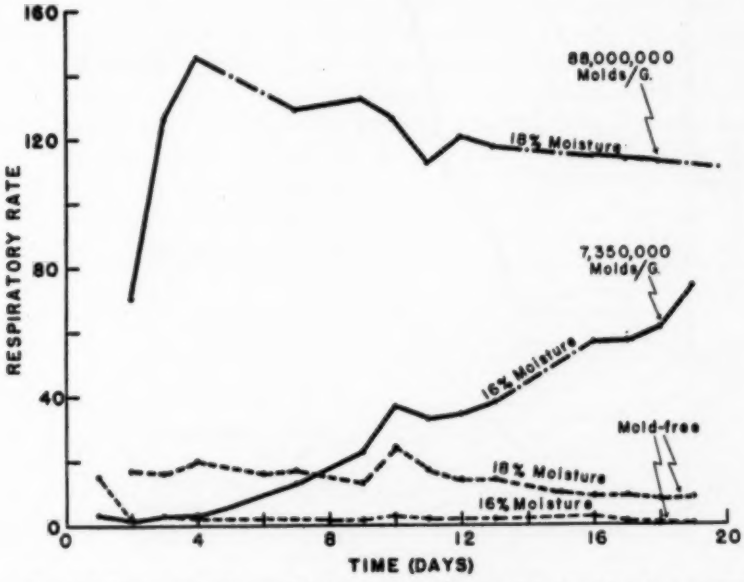

Fig. 1. Influence of mold contamination on the respiratory rates of white wheat at 35°C. and moisture contents of about 16 and 18%. After surface-sterilization, subsamples were conditioned to each moisture level with 1.0% sodium hypochlorite solution and with a mold inoculum, respectively. The aeration rate was 1.5 liters/100 g./24 hours. Respiratory rate is expressed as mg. carbon dioxide per 100 g. of dry matter per 24 hours.

S-shaped curves obtained in previous studies with various grains. The marked differences between the mold-free and mold-infected samples are shown by the mean respiratory rates for the 15th to 19th day of storage which follow:

Mean Respiratory Rate (mg. CO_2/24 Hours/100 g. Dry Matter) from the 15th to the 19th Day Inclusive for Wheat at Various Initial Moisture Values

Moisture %	14.9	16.0	18.0	20.2	24.2	27.7	30.8
Mold-free	1	1	6	55	179	549	265
Moldy	2	23	56	1404	1694	1639	1608

The sample stored at 27.7% moisture which was presumably mold-free is of particular interest. After 10 days, the respiration of this sample began to increase and a mold count at the end of the trial

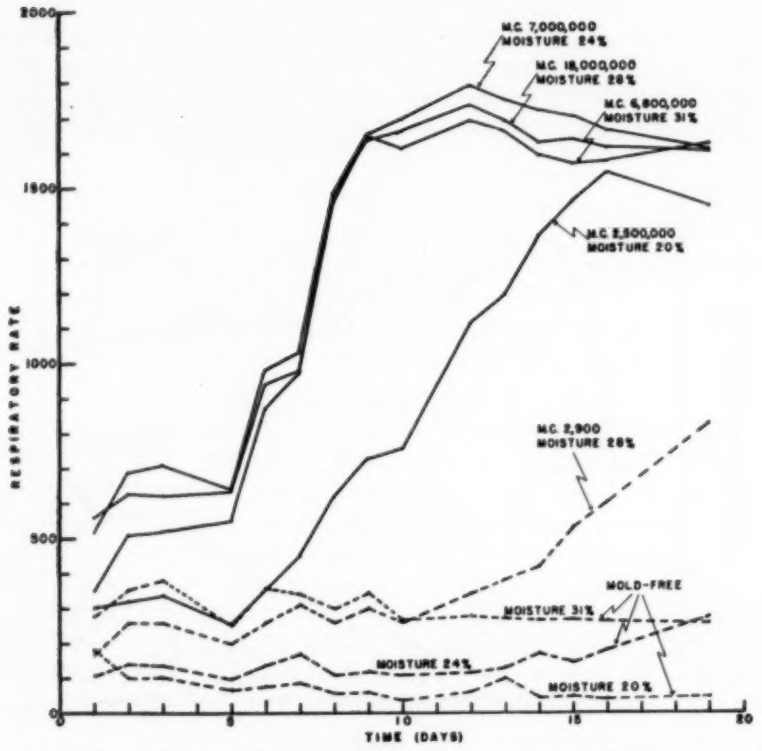

Fig. 2. Influence of mold contamination on the respiratory rates of white wheat at 35°C. and moisture contents of about 20, 24, 28, and 31%. After surface-sterilization, subsamples were conditioned to each moisture level with sterile water and with mold inoculum, respectively. Respiratory rate is expressed as mg. carbon dioxide per 100 g. of dry matter per 24 hours. During the first 5 days the aeration rate was 1.5 liters/100 g./24 hours; on the sixth day the aeration was increased to 2.0 liters and on the eighth day to 3.9 liters (the maximum available with the apparatus). These rates were not adequate to prevent exhaustion of the oxygen in the samples at the three highest moisture levels.

revealed the presence of a light infection (2900 spores per g.). In general, the respiratory rate of mold-free grain increased appreciably as the moisture content was raised, but the increase was relatively insignificant when compared to that of mold-infected wheat. The respiration of the samples stored at initial moisture contents of 24.2% and higher was so rapid that, even with the maximum aeration rate which could be provided (3.9 l. per 100 g. per 24 hours), the daily oxygen supply was virtually exhausted after the first 9 days of the trial. It is not surprising, therefore, that the samples at initial moisture contents of 24.2, 27.7, and 30.8% exhibited very similar rates after the first few days. During the last few days, the respiration of the sample

at 20.2% moisture was also somewhat limited by the oxygen supply. The respiration rates for these samples, therefore, do not represent the true potential values for samples at the four highest moisture levels which were employed.

Viability. The viability of wheat stored at moisture contents of 14.9, 16.0, and 18.0% decreased with increasing moisture content; at each moisture level the samples contaminated with molds were of lower viability than those which were mold-free (Table I). At moisture levels of 20.2% and higher, all the samples were dead.

It appears that the storage of wheat at high moisture contents and 35°C. can reduce viability in the absence of molds. This indicates that between 18 and 31% moisture, wheat may carry on a limited metabolism which leads to deterioration and death of the embryo through the depletion of certain essential metabolites or the accumulation of some substance (s) in toxic concentrations.

TABLE I

MOLD COUNT, NATURE OF MOLDS, AND VIABILITY OF WHEAT STORED FOR NINETEEN DAYS AT 35°C.

Initial Moisture Content	Condition of Wheat	Total Mold Count	Principal Internal Molds Present		Viability of Wheat After Storage[1]
%		(colonies/g.)		%	%
14.9	Mold-free	0	*Aspergillus glaucus*		70
	Moldy	83,000	*Aspergillus flavus*		41
16.0	Mold-free	0	———		30
	Moldy	7,350,000	*Aspergillus glaucus*		
			Aspergillus flavus		10
			Aspergillus ochraceus		
			Penicillium		
18.0	Mold-free	0	———		8
	Moldy	88,800,000	*Aspergillus flavus*		
			Aspergillus ochraceus		2
20.2	Mold-free	22	*Oospora*	64.0	
			Aspergillus flavus	18.0	0
			Aspergillus glaucus	6.0	
			Aspergillus niger	6.0	
	Moldy	2,560,000	*Aspergillus flavus*	100.0	0
24.2	Mold-free	36	*Oospora*	100.0	0
	Moldy	7,000,000	*Aspergillus flavus*	100.0	0
27.7	Mold-free[2]	2,900	*Oospora*	99.0	
			Aspergillus niger	1.0	0
	Moldy	18,000,000	*Aspergillus flavus*	92.0	
			Aspergillus candidus	8.0	0
30.8	Mold-free	6	*Hormodendrum*	100.0	0
	Moldy	6,800,000	*Aspergillus flavus*	90.0	
			Aspergillus glaucus	4.0	0
			Penicillium	3.0	
			Aspergillus terreus	1.5	
			Apergillus ochraceus	1.5	

[1] Initial germination was 95%.
[2] Slight accidental contamination.

Mold Count and Species Distribution. The mold data in Table I show that the numbers of mold spores in the mold-infected samples increased with increasing moisture contents of 14.9 to 18.0%; the principal species present were *Aspergillus glaucus, A. flavus,* and *A. ochraceus.* At higher moisture contents, the mold count fluctuated erratically; although *A. flavus* was predominant, some species were present in addition to those found in the samples of lower moisture content. No significance can be attached to the mold count for the samples stored at initial moistures of 20.2% and higher because of the inadequate aeration which these samples received.

Fat Acidity and Reducing and Nonreducing Sugars. The fat acidity and sugar values in Table II show that the changes in these properties upon storage are associated with the growth of microorganisms rather than with the metabolic activity of the wheat itself. With increasing mold contamination, the fat acidity and reducing sugar content increased while the nonreducing sugar content decreased.

TABLE II

EFFECT OF MOISTURE CONTENT AND MOLD INFECTION ON THE CHANGES IN FAT ACIDITY AND SUGAR CONTENT OF WHEAT UPON STORAGE FOR NINETEEN DAYS AT 35°C.

Moisture			Fat	Sugars	
Initial	Final	Condition	Acidity[1]	Reducing[2]	Nonreducing[3]
10.5		Original sample	14.1	34	201
14.9	14.6	Mold-free	13.7	47	227
	15.0	Moldy	16.8	52	224
16.0	15.9	Mold-free	14.7	44	218
	16.4	Moldy	37.6	51	181
18.0	18.0	Mold-free	12.1	39	229
	19.1	Moldy	77.0	84	134
20.2	22.0	Mold-free	15.0		
	26.5	Moldy	154.0		
24.2	25.8	Mold-free	16.3		
	31.8	Moldy	169.4		
27.7	29.7	Mold-free[4]	24.9		
	34.8	Moldy	174.4		
30.8	32.8	Mold-free	12.3		
	37.7	Moldy	173.5		

[1] Expressed as mg. KOH per 100 g. wheat, dry basis.
[2] Expressed as mg. of maltose per 10 g. wheat, dry basis.
[3] Expressed as mg. of sucrose per 10 g. wheat, dry basis.
[4] Some accidental mold contamination.

Discussion

The results of these studies support the view expressed in previous papers in this series that the increases in respiration and fat acidity, and the decreases in nonreducing sugars which occur when wheat and other cereal grains are stored at moisture contents exceeding 14.5 to 15.0% are largely due to the growth of molds normally present on and within the seed. So far as the authors are aware, this is the first time that wheat of high viability and free of molds has been employed in storage trials. Several years ago, in a preliminary attempt to estimate the inherent respiration of wheat independent of that due to molds, Milner and Geddes treated wheat with thiourea to inhibit mold growth (6). Molds were only partially inhibited but the respiration and fat acidities of the samples treated with thiourea were markedly lower than those for the untreated wheats. The present experiments provide a clear-cut distinction between deteriorative processes inherent in the seeds themselves and those produced by the normal microflora on and within the seed. While increased moisture somewhat augmented the respiration, fat acidity, and changes in the sugar contents of mold-free wheat, the differences were very slight compared with those which occurred when molds were present. The direct and striking experimental evidence obtained in these studies establishes the importance of molds in the deterioration of stored grain.

Acknowledgment

The authors are indebted to T. A. Grahek for making the mold assays.

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AN ALPHA-AMYLOGLUCOSIDASE THAT PRODUCES BETA-GLUCOSE [1]

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ABSTRACT

An amylase preparation from *Aspergillus niger* NRRL 599 catalyzed the hydrolysis of starch and maltose to produce beta-glucose. Repeated fractionations of the sample increased the ratio of saccharogenic activity to dextrinizing activity. However, some dextrinizing activity remained in the most highly purified samples. Glucose was the only reducing sugar obtained in the hydrolysate when this enzyme was used.

Hydrolysis of both starch and maltose by this enzyme produced solutions which exhibited rising mutarotation.

The presence of an amyloglucosidase or glucoamylase has been reported in the extracts of *Aspergillus niger* NRRL 330 (1, 3) and *Rhizopus delemar* (10). It has been shown that the amyloglucosidase of *Aspergillus niger* NRRL 330 acts like a beta-amylase in breaking off glucose from the nonreducing ends of the starch chain (3).

An amylase preparation from *Aspergillus niger* NRRL 599 yielded large amounts of glucose as an end-product in the hydrolysis of starch. It seemed of interest to determine whether glucose was the only reducing product in the hydrolysate. Because the enzyme also possessed the ability to digest starch rapidly to the extent that it yielded only a red color with iodine, it seemed important to determine whether the dextrinizing action could be removed by fractionation, or partial inactivation with acid.

Previous investigators have shown that the hydrolysates from some of the beta-amylases exhibit rising, or beta-, mutarotation (5, 9). The hydrolysates from the other amylases generally exhibit falling muta-

[1] Manuscript received July 10, 1953.
Funds for this research were made available by the Rutgers University Research Council. Samples of the amylase preparation were provided by the Takamine Laboratory, Inc., Clifton, N. J.
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rotation. It seemed of interest to determine the type of mutarotation encountered in the hydrolysates produced by *Aspergillus niger* NRRL 599. If the enzyme catalyzed the hydrolysis of starch to beta-glucose, then the solution should show rising mutarotation. If alpha-glucose was formed, then there should be falling mutarotation.

Materials and Methods

The amylase preparation supplied by the Takamine Laboratory, Inc., was obtained by growing the mold on a solid medium, and then percolating the medium with water. The percolate was partially dialyzed against running tap water, and then precipitated with an organic solvent.

Three types of measurement were employed as indexes of amylase activity. These were the saccharogenic activity, the "glucose-direct" activity, and the amyloclastic activity.

The term saccharogenic activity refers to the measurement of total reducing groups in the hydrolysate. The analyses were carried out at 40°C. on a 2% solution of Takamine soluble starch in the presence of 0.02*M* acetate at pH 4.5. An iodine-thiosulfate method (8) was employed, and the results calculated as mg. of glucose per mg. of enzyme preparation, even though maltose and other sugars might contribute to this reducing measurement.

The "glucose-direct" activity was determined on the same hydrolysates used for the measurement of saccharogenic activity. Glucose was determined in the presence of other reducing sugars by the method of Zerban and Sattler (11), and the results expressed as mg. of glucose per mg. of enzyme preparation.

Amyloclastic activity is a measure of the amount of starch hydrolyzed to the extent that the solution gave a red color with iodine. These experiments were carried out under conditions similar to those of the two previous measurements, except that the starch concentration was 1% and the acetate concentration was 0.1*M*. Results are expressed as mg. of starch hydrolyzed by 1 mg. of enzyme preparation in 30 minutes.

In those experiments in which the activity of an altered enzyme was compared to that of the original, the measurements were made in the early stages of the hydrolysis; that is, the time when the glucose formed is directly proportional to the amount of enzyme present.

Maltase activities were carried out under the same conditions of temperature and pH as the measurements of saccharogenic activity. A 0.4% solution of maltose hydrate (Pfahnstiehl) was used as the sub-

strate, except in an experiment on mutarotation where it was 1%. Because the glucose formed was not exactly a linear function of the amount of enzyme, a curve similar to that of Gates and Kneen (2) was developed. The values for this curve corresponded almost exactly to those found by Gates and Kneen. Results were calculated as mg. of glucose formed per mg. of enzyme preparation.

The experiments on mutarotation were all carried out at 25°C. ±0.5°C. in the presence of 0.02*M* acetate buffer at pH 4.5. A Schmidt-Haensch polarimeter fitted with 2-dcm. tube and sodium light was used. Readings were taken at the same time the aliquots were removed for reducing group analysis.

The $[\alpha]^{25}_D$ of the original starch was +196° while that of the maltose hydrate was +131°.

The velocity constant for the mutarotation of glucose was determined at 25° at pH 4.5. This first order constant is referred to as k_2 in the calculations.

Results

Comparison of Saccharogenic, "Glucose-Direct", and Amyloclastic Activities upon Fractionation of the Enzyme. Table I shows the data for the hydrolysis of starch catalyzed by the enzyme from *A. niger* NRRL 599. The close agreement between the saccharogenic activity (total reducing groups), and the "glucose-direct" measurements indicates the absence of reducing sugars other than glucose even in the early stages of the hydrolysis. The presence of maltose or other reducing polysaccharides would have caused the values listed under saccharogenic activity to be higher than those under "glucose-direct" as was found in the early experiments with the enzyme from *Rhizopus delemar* (10).

TABLE I

ACTION OF AMYLASE FROM ASPERGILLUS NIGER 599 ON STARCH

Hydrolysis Time	Glucose per mg. Dry Enzyme Preparation	
	Glucose-Direct	Saccharogenic
	mg.	*mg.*
30 minutes	8	7
60 minutes	15	15
120 minutes	32	32
240 minutes	56	54
24 hours	102	97

The enzyme showed considerable amyloclastic activity and measurable maltase activity. A solution of the enzyme was fractionated with ammonium sulfate in an effort to free the amyloglucosidase of amyloclastic or Wohlgemuth activity. Changes in the various activities were followed by calculating ratios; thus, A/S represents the ratio of amyloclastic to saccharogenic activity, and S/M is the ratio of saccharogenic to maltase activity. The data for a complete fractionation of the enzyme given in Table II show some changes in ratios. The

TABLE II

ACTIVITIES OF FRACTIONATED SAMPLE OF ENZYME FROM ASPERGILLUS NIGER 599

Treatment with $(NH_4)_2SO_4$	Amyloclastic (A) Starch Hydrolyzed / Starting Material	Saccharogenic (S) Glucose / Starting Material	Maltase (M) Glucose / Starting Material	A/S	S/M
	mg.	*mg.*	*mg.*		
None	30.0	7.5	5.0	4.0	1.5
2/3 Sat.	2.3	0.35	0.17	6.5	2.0
4/5 Sat.	12.3	2.46	1.58	5.0	1.6
Sat.	8.3	3.15	2.32	2.6	1.4

amyloclastic activity exhibited some tendency to be concentrated in the first fraction. Particular attention was given to the last fraction where the A/S ratio was lowest. Attempts were made to free this portion of amyloclastic activity by refractionation with ammonium sulfate, followed by fractionation with ethanol. Neither of these technics produced any significant change in the ratios. Furthermore, glucose was the sole reducing product in the hydrolysate, regardless of which fraction was used as the enzyme.

Phillips and Caldwell found that the low pH treatment used in the experiments with *A. niger* NRRL 330 (3) did not inactivate the amyloclastic activity of the crude precipitate from *Rhizopus delemar* (10). However, treatment of the purified material at 5°C. at pH 2.4 for 9 days resulted in the complete destruction of the Wohlgemuth activity. The enzyme system from *A. niger* NRRL 599 proved much more stable. A sample of the enzyme was fractionated twice with ammonium sulfate, and once with ethanol. No change in A/S ratio could be observed even after 1 month's exposure to pH 2.4 at 5°C.

In experiments with bentonite adsorption at 5°C. and pH 2.4, the A/S ratio was found to be unchanged. Furthermore, the loss of enzyme was so great that this technic seemed to hold little promise.

Phenyl mercuric chloride has been used to inactivate at least one

of the amylases (11). It had no effect on the amylase from *A. niger* NRRL 599.

Nitrite in acid solution has been used to inactivate amylases (6, 7, 11). A sample of the original enzyme preparation was dissolved in acetate buffer at pH 4.5 and brought to 40°C. Enough sodium nitrite was added to make the solution 0.16*M* with respect to this reagent. Aliquots were removed at specified intervals, and mixed with phosphate buffer at pH 7.2 to stop the action of the nitrite. The samples were then measured for all three types of activity. The results are shown in Table III. The changes in A/S ratio are so small that they cannot be considered to be significant.

TABLE III

EFFECT OF NITRITE IN ACID MEDIUM ON THE ACTIVITIES[1] OF ENZYME FROM ASPERGILLUS NIGER 599

Time of Treatment in Hours	Amyloclastic Activity (A)	Saccharogenic Activity (S)	Maltase Activity (M)	A/S	S/M
0	30	7.50	4.94	4.0	1.5
1	29	4.80	2.88	4.2	1.7
2	29	4.63	2.55	4.3	1.8
4	18	3.98	2.34	4.5	1.7
7	17	3.60	2.12	4.7	1.7

[1] Activity per mg. of enzyme preparation in 30-minute hydrolysis.

The crude enzyme preparation was very stable in the presence of large excesses of silver, cupric, ferric, mercuric, phosphotungstic, and phosphomolybdic ions. One hundred milligrams of the material were not inactivated by 2 ml. of a 2% solution of sodium lauryl sulfonate. Even after fractionation the enzyme was markedly stable in the presence of heavy metal ions.

Mutarotation Studies. In the mutarotation experiments there were certain limitations imposed by the nature of the work. Because the starch solution became cloudy around 45% theoretical hydrolysis, it was necessary to restrict the studies to the early portion of the curve. While it was important for the kinetic studies to keep the ratio of enzyme to substrate low enough to be able to measure the k_1 of the zero order reaction, it was even more important to carry out the hydrolysis as rapidly as possible. An extremely slow rate of hydrolysis would have resulted in the mutarotation keeping pace with the formation of the glucose.

Ten milliliters of a sample of the enzyme which had been fractionated twice with ammonium sulfate were allowed to act on 100 ml.

of a 2% soluble starch solution at pH 4.5 and 25°C.±0.5°C. The rotation and glucose content of the hydrolysate were measured at the specified intervals.

Calculated rotations were then computed from the glucose determinations for each point. These calculations were based on the amount of glucose formed, and the amount of starch remaining. The $[\alpha]^{25}_D$ for glucose was taken as +52.6°, and that of starch as +196°. The observed and calculated rotations are plotted in Fig. 1. The observed rotations are in each case lower than the calculated rotations. In the same figure are plotted the calculated rotations, assuming: 1) that the newly formed glucose is alpha-glucose with an $[\alpha]^{25}_D = +113°$; or 2) that the newly formed glucose is beta-glucose with an $[\alpha]^{25}_D + 19°$. The measured rotations are much closer to the calculated rotations for beta-glucose. This indicates that beta-glucose is formed, and then undergoes mutarotation.

Mutarotation begins to take place as soon as some glucose is formed. It seemed necessary to take this into consideration in the calculated rotations. The hydrolysis of the starch follows a zero order reaction in the range studied here. The velocity constant was found to be 0.136 mg. glucose per minute. The mutarotation of glucose is a first-order reaction. At the temperature used in these experiments, the velocity constant for the reaction, k_2, was found to have the value 0.0342 per minute. The entire reaction may be represented as: $A \xrightarrow{k_1} B \xrightarrow{k_2} C$, where A represents starch, B the newly formed alpha- or beta-glucose, and C the mutarotated glucose. The amount of B remaining at any time may be calculated from the equation: $B = (k_1/k_2)(1 - e^{-k_2 t})$

The calculated rotations are compared with the measured rotation in Fig. 2. While the lower curve, in which B is taken as beta-glucose, does not exactly coincide with the measured rotation, there is sufficiently close agreement to state that there is beta-mutarotation. The curve in which B is calculated as alpha-glucose differs quite markedly from the measured rotation.

In both Figs. 1 and 2, rotation of the unhydrolyzed starch was computed by multiplying the measured rotation of the original 2% solution by the fraction remaining. The validity of using such a calculation was shown in the following way.

The enzyme concentration was reduced to one-fortieth of that used in securing the data presented in Figs. 1 and 2. Thus k_1 was so small that the mutarotation almost kept pace with the hydrolysis. When 3.75 mg. of glucose had been formed the measured rotation was

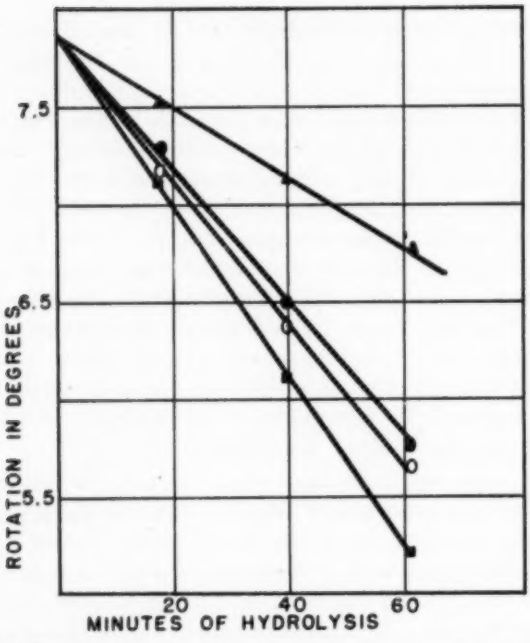

Fig. 1. Hydrolysis of starch by an amylase preparation from Aspergillus niger NRRL 599. Measured and calculated rotations: ○, measured rotation; ▲, calculated rotation if glucose formed is alpha-glucose; ●, calculated rotation if glucose formed had completely mutarotated; ■, calculated rotation if glucose formed is beta-glucose.

+6.92°. The calculated rotation at this point was +6.90°, using +52.6° as the $[\alpha]^{25}_D$ for the mutarotated glucose.

The hydrolyses were repeated using maltose hydrate, $[\alpha]^{25}_D = +131°$, as the substrate. The problem is slightly complicated by the fact that two glucose molecules are set free for each maltose which is split. Fortunately, the normally free aldehyde end in maltose has about the same ratio of *alpha* to *beta* as is present in mutarotated glucose. Therefore mutarotation will take place only at the aldehyde group set free in the hydrolysis. When the data were plotted, there was obtained a curve similar to that of Fig. 1. There is definite beta-mutarotation. The data were not subjected to the kinetic treatment used in the starch experiments because the hydrolysis of maltose did not follow the simple kinetics of the starch hydrolysis.

Discussion

The glucose-producing amylase of *A. niger* NRRL 599 seems to resemble the amylase system of *A. niger* NRRL 330 and *Rhizopus*

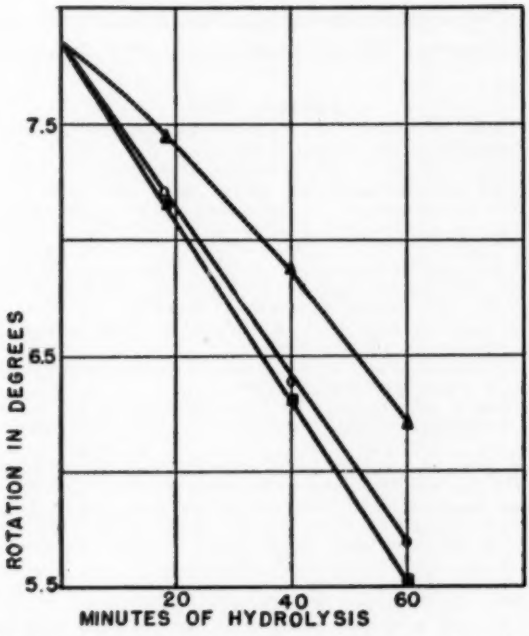

Fig. 2. Hydrolysis of starch by an amylase preparation from Aspergillus niger NRRL 599. Measured and calculated rotations with correction for mutarotation: O, measured rotation; ▲, calculated rotation if glucose formed is alpha-glucose; ■, calculated rotation if glucose formed is beta-glucose.

delemar. However, it has not been possible to free the amyloglucosidase of amyloclastic activity. A single fractionation can produce a preparation with an A/S value of 2.6 to 2.8 but repeated fractionations did not lower this ratio. These results lead to the suggestion that this amyloglucosidase may also have amyloclastic activity.

While the stability of the enzyme in the presence of heavy metal ions is of interest, it is not unusual. Some of the pectin enzymes are remarkably stable under these conditions (4).

Beta-mutarotation has been shown by the hydrolysates. While this beta-mutarotation is postulated on the basis of fairly small differences in rotations, these differences are quite definite. The results have been repeated several times. The curves in Fig. 2 show that large differences in rotation could not be expected. The concentrations of glucose formed are low, and there is continuous mutarotation. Another point in favor of beta-mutarotation is the large difference between the curve for measured rotation and that calculated for glucose formed as alpha-glucose. Admittedly, it has not been shown that there is a com-

plete absence of alpha-mutarotation, but it has been demonstrated that the rising mutarotation is predominant with this enzyme.

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COMMUNICATION TO THE EDITOR

Nonconformity of Purified Gluten to the Maxwell Equation

Dear Sir:

Dr. Udy's[1] statements that "the work of Schofield and Scott Blair indicated that the purified gluten might closely simulate a Maxwell element" and that "ordinary wheat-flour dough obeys the Maxwell relationship" are misleading.

In the paper quoted by Dr. Udy[5] we commented on the close resemblance between the behavior of dough and gluten and made it clear that neither material obeys Maxwell's equation. In the three earlier papers of the series,[2,3,4] not quoted by Dr. Udy, this is even more apparent. At the beginning of the first of these papers, we described dough as a "plastic" material and went straight on to emphasize that for such materials, relaxation time is a quantity which is not a constant, but which varies according to the stress. In the third paper, we introduced into the Maxwell equation a term $d\alpha/dt$ and pointed out that, except in the limit when the dough has been undisturbed for some time, this term is large and cannot be ignored. During one of the relaxation experiments quoted, the relaxation time varied from 126 to 1980 seconds, which hardly suggests a constant value.

We cannot agree that "the fact that the 'purified' gluten does not follow this [simple Maxwellian] relationship would lend supporting evidence to the hypothesis of a carbohydrate-protein interaction" though of course our work in no way invalidates such a hypothesis.

Our sole purpose in writing this letter is to correct the mistaken notion that we ever believed that flour dough obeys Maxwell's equation, except under limiting conditions.

January 26, 1954.

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BOOK REVIEWS

Storage of Cereal Grains and Their Products. Edited by J. A. Anderson and A. W. Alcock, 600 pp., American Association of Cereal Chemists, Monograph Series, Volume II. 1954. Price $11.00.

The eleven chapters of this monograph cover the entire range of detailed considerations suggested by its title. Each chapter is written by a specialist or team of specialists who has, or have, unusual competence in and familiarity with the subject matter of the chapter. The treatment accorded these respective fields is complete, orderly, and readable. Thus the data and the technical and scientific observations recorded in over 1,000 published papers and books are interwoven into a logical and concise pattern in the text of the appropriate sections of the book. The manner of presentation is suggested by the eleven chapter headings which are as follows: — I. Moisture and its measurement, by I. Hlynka and A. D. Robinson; II. Chemical, physical, and nutritive changes during storage, by Lawrence Zeleny; III. Microflora, by G. Semeniuk; IV. Respiration and heating, by Max Milner and W. F. Geddes; V. Insects, by R. T. Cotton; VI. Rodents, by D. A. Spencer; VII. Country storage of grain, by H. J. Barre; VIII. Terminal elevator storage, by J. E. Bailey; IX. Drying of grain, by W. V. Hukill; X. Flour storage in bulk, by R. E. Hamilton, C. J. Lynde, and R. K. Larmour; XI. Packaging and storage of cereal products, by C. A. Southwick.

The scope and adequacy of presentation is illustrated by the first chapter, on moisture and its measurement. Doctors Hlynka and Robinson open with a discussion of certain basic considerations including adsorption, the forces involved, relations of chemical structure, water sorption isotherm, isotherms for cereals, and bound water. This is followed by a detailed discussion of the determination of water content, including the use of ovens; distillation, chemical, and electrical methods, and finally of primary reference methods against which secondary properties such as electrical conductance and capacitance may be calibrated.

Obviously many of the fundamental properties here presented find application in later chapters involving technological considerations that enter into the practices which can be followed to advantage in industry.

The more fundamental considerations presented in the first six chapters are balanced there and in the latter chapters of the monograph by very practical discussions of the storage of grain and grain products, and the drying of grain. Thus one finds useful information concerning materials used in the construction of granaries and elevators, and of machines and equipment available for drying and treating grain and cereal products in the interests of increasing and improving the storage life of such materials. Practical protection against damage by insects and rodents also receives attention.

A monograph so carefully and logically planned, and with the details organized by such competent authors, cannot fail to be of substantial service to a large audience, including those concerned not alone with the more fundamental aspects involved but also with commercial practice in the handling and warehousing of the common cereals and their products.

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Soil and Fertilizer Phosphorus in Crop Nutrition. Vol. IV of **Agronomy,** ed. by W. H. Pierre and A. G. Norman. xvi + 492 pp. Academic Press, Inc.: New York, 1953. Price $9.00.

The information presented at a symposium on phosphorus held at the University of Illinois in 1952, plus some additional material, has been assembled in this book, which is the work of twenty-nine authors. It is without question the most complete assemblage of information on soil and fertilizer phosphorus which has ever been brought together. Its fifteen chapters cover a range of topics from

the "Physiology and Biochemistry of Phosphorus in Green Plants," Chapter I, to "World Phosphorus Fertilizer Production, Consumption, and Potential Needs," Chapter XV.

The topics discussed in this book can be classed into a few main groupings. Chapters I and II have to do particularly with soil-plant relationships, including the functions of phosphorus in plant nutrition and the absorption of phosphorus by plants in relation to crop growth.

Chapters III, IV, and V deal largely with soil phosphorus: the forms, amounts, reactions, and properties of inorganic and organic phosphates in acid, neutral, alkaline, and calcareous soils. Chapter VI is somewhat related in topic to the preceding three in that chemical soils tests for evaluating soil phosphorus are discussed.

The next six chapters are concerned largely with phosphate fertilizers; Chapters VII, VIII, and IX deal with some of the aspects and relationships of phosphorus added to soils; Chapters X, XI, and XII are confined largely to problems of phosphate fertilizers alone, their laboratory evaluation, our domestic phosphate deposits, and technical and economic factors influencing phosphate fertilizer production in the United States.

In the last three chapters the more general problems of phosphorus fertilizer use are considered in relation to the phosphorus status of soils, crop requirements, and potential fertilizer needs in the United States and in other parts of the world.

This book is an outstanding addition to the literature on soil and fertilizer phosphorus. Old and new beliefs and findings have been brought together as in no other single source. Contributions of various workers in the field have been freely cited, and the compilation of the bibliography alone has been a major accomplishment. This is a technical publication and thus is not light reading. It is not a book for the uninformed and will probably find its main use as a reference work. However, it will prove to be an invaluable source of information on phosphorus for soils people, agronomists, and professional fertilizer personnel.

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Cereal Chemistry

EDITORIAL POLICY

Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, or fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

Cereal Chemistry gives preference to suitable papers presented at the Annual Meeting of the American Association of Cereal Chemists, or submitted directly by members of the Association. When space permits, papers are accepted from other scientists throughout the world.

The papers must be written in English and must be clear, concise, and styled for **Cereal Chemistry.**

Manuscripts for publication should be sent to the Editor in Chief. Advertising rates may be secured from and subscriptions placed with the Managing Editor, University Farm, St. Paul 1, Minnesota. Subscription rates, $11.00 per year. Foreign postage, 50 cents extra. Single copies, $2.50; foreign, $2.60. Back issues, $3.00.

SUGGESTIONS TO AUTHORS

General. Authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* 6:1–22. 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

Editorial Style. A.A.C.C. publications are edited in accordance with *A Manual of Style,* University of Chicago Press, and *Webster's Dictionary.* A few points which authors often treat wrongly are listed below:

Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°–10°C.). Place 0 before the decimal point for correlation coefficients ($r = 0.95$). Use * to mark statistics that exceed the 5% level and ** for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., A/(B + C). Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

For more detailed information on manuscript preparation see *Cereal Chem.* **30:** 351–352 (1953).

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